STUDY OF DOUGLAS-FIR BARK DEVELOPMENT AND CHARACTERIZATION OF ITS CORK COMPONENT

MARIA SOFIA QUINHA CARDOSO

SCIENTIFIC ADVISORS: Ph.D Helena Margarida Nunes Pereira
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THESIS PRESENTED TO OBTAIN THE DOCTOR DEGREE IN FORESTRY ENGINEERING AND NATURAL RESOURCES

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INSTITUTO SUPERIOR DE AGRONOMIA
Universidade de Lisboa

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ABSTRACT

Douglas-fir (*Pseudotsuga menziesii*) is a valued timber conifer providing long sawnwood components, recognized for its fast growth, natural regeneration and adaptability. Original from North America, it was planted in Europe on approximately 550 thousand ha including Portugal, where its potential area is estimated at 250,000 ha.

The Douglas-fir bark has a high cork proportion and its potential use was already discussed in early studies.

This research was made on mature trees from the north and central mountains of Portugal. Stem development regarding heartwood, sapwood and bark was analyzed as well as bark structural development. Heartwood represented 49 % of the cross section in the lower stem and sapwood width was on average 75 mm at stem base, both decreasing upwards. The bark was approximately 3 cm thick at stem base, where the rhytidome corresponded to 84 % of the bark, including nearly 50 % of cork, and decreased to 3–5 mm at the top. The cork is not continuous within the rhytidome, and the layers are interspersed with phloem.

The cork was studied regarding structural and anatomical features. It was characterized by extensive areas of crushed cells, making up a very compact structure, with patches of uncompressed cork. In the uncompressed regions the cellular dimensions were: prism height 55 μm, base area 1388 μm^2 and 1.3 μm cell wall thickness.

Chemical composition of Douglas-fir bark varies with age: at 45, 30 and 17 years of age, the bark contained respectively 25.4 %, 2.6 % and, 0.9 % suberin. Cork and phloem differ in extractives (49.8 % vs. 17.0 %), suberin (30.1 % in cork vs. not present in phloem) and hemicelluloses composition regarding arabinose content (25.3 % vs. 4.8 % of monosaccharides).

Douglas-fir bark proved to be an interesting feedstock for biorefineries while a valorization targeting cork should consider using the lower stem parts of mature trees.

Keywords: Douglas-fir; Bark; Cork; Anatomy; Chemistry

RESUMO

Pseudotsuga menziesii (Douglas-fir) é uma conífera produtora de madeira de alta qualidade para usos estruturais, com crescimento rápido, boa regeneração natural e grande adaptabilidade. Original da América do Norte foi introduzida na Europa, onde ocupa 550 mil ha, e em Portugal, onde se estima que possa ser plantada em 250,000 ha. A casca de pseudotsuga apresenta uma proporção considerável de cortiça cuja utilização foi já considerada.

Neste estudo utilizaram-se árvores adultas das regiões norte e centro de Portugal. Analisou-se o desenvolvimento do tronco considerando cerne, borne e casca. Em média, a base das árvores apresenta 49 % de cerne e 75 mm de espessura de borne, ambos diminuindo para o topo. A casca na base do tronco possui aproximadamente 3 cm de espessura, dos quais 84 % é ritidoma com quase 50 % de cortiça, diminuindo até 3-5 mm de espessura no topo. No ritidoma as camadas de cortiça apresentam-se intercaladas com floema.

O estudo estrutural e anatómico da cortiça mostrou a existência de áreas extensas com células de cortiça esmagadas, formando uma estrutura compacta com zonas de células não compactadas. Nestas, as células de cortiça apresentam 55 μm de altura de prisma, 1388 μm^2 de área de base e paredes celulares com 1,3 μm de espessura.

A composição química da casca de pseudotsuga varia com a idade: aos 45, 30 e 17 anos, a casca contem respectivamente 25,4 %, 2,6 % e, 0,9 % de suberina. A cortiça e floema diferem no teor em extractivos (49,8 % vs. 17,0 %), suberina (30,1 % cortiça vs. ausente no floema) e composição de hemiceluloses principalmente na proporção de arabinose (25,3 % vs. 4,8 % em monossacáridos).

A casca de pseudotsuga demonstrou ter potencial para biorrefinarias e a parte inferior do tronco de árvores adultas poderá ser considerada como potencial fornecedora de cortiça.

Palavras-chave: Pseudotsuga; Casca; Cortiça; Anatomia; Química

RESUMO ALARGADO

A *Pseudotsuga menziesii* (Douglas-fir) é considerada uma das espécies de coníferas comercialmente mais importante no mundo. Produtora de madeira de grande qualidade para usos estruturais é reconhecida pelo seu rápido crescimento, boa regeneração natural e grande adaptabilidade. É uma espécie natural da América do Norte e foi introduzida na Europa onde ocupa aproximadamente 550 mil ha, e também nas regiões montanhosas de Portugal, onde se estima que possa ser plantada em cerca de 250,000 ha. Apesar do rápido crescimento e do seu valor como espécie madeireira, existem poucos trabalhos em relação à sua caracterização no nosso país.

Neste estudo utilizaram-se amostras de 20 árvores adultas das regiões norte e centro de Portugal (Serra da Cabreira e Serra da Estrela), caracterizando-se o seu crescimento através da análise do desenvolvimento do cerne, borne, casca e dos anéis de crescimento. A taxa de crescimento foi de 7,1 e 6,6 mm por ano para árvores com 45-50 anos de idade. A proporção de cerne é substancial, em média correspondendo a 49 % da área seccional, na base do tronco das árvores, decrescendo esta proporção para o topo. Estimativas decorrentes deste estudo indicam que o cerne começa a formar-se aos 8-9 anos de idade da árvore, formando-se 0,7-0,9 anéis por ano. Por seu lado, o borne na base das árvores mede, em média, 75 mm de espessura, decrescendo para o topo. É na base das árvores que a casca é mais espessa, em média 26-27 mm, representando 15 % da área seccional total e diminuindo para 3-5 mm de espessura no topo das árvores. Sendo esta uma espécie com grande potencial para o nosso país e tendo em conta as condições silvícolas dos locais amostrados neste trabalho, as árvores apresentam troncos com aptidão para a produção de componentes de madeira de grandes dimensões que poderão ser utilizados para fins estruturais na construção.

A casca foi estudada em termos estruturais e anatómicos incluindo uma análise detalhada do arranjo dos tecidos, biometria celular, proporção de tecidos no floema e desenvolvimento do ritidoma. Verificou-se a existência de uma relação entre a dimensão das células, proporção de tecidos no floema e a idade; o efeito da posição em altura no tronco é estatisticamente significativo para o comprimento das células crivosas e, comprimento e espessura da parede celular dos fibro-esclereídos com diminuição da base para o topo. A espessura do ritidoma aumenta com a idade cambial: na base das árvores (45-50 anos de idade cambial), a casca inclui um ritidoma com cerca

de 3 cm de espessura correspondendo a 84 % da casca, com 5-8 peridermes, contendo quase 50 % de cortiça. As células de cortiça apresentam paredes celulares finas e encontram-se orientadas radialmente com a presença de células lenhificadas com paredes espessas associadas ao aumento das camadas de felema. Nas peridermes jovens a presença de células de felema com lúmens vazios e paredes celulares finas e suberizadas ocorre aos 25-30 anos de idade cambial.

Considerando a valorização desta casca tendo em conta a sua componente de cortiça, foi também feito o estudo do desenvolvimento da casca ao longo do tronco das árvores do ponto de vista estrutural e químico em relação com a idade cambial. Esta espécie apresenta uma casca espessa que com a idade desenvolve um ritidoma com diversas peridermes contendo uma proporção substancial de cortiça. A valorização da casca será uma vantagem que poderá ser incluída na exploração da madeira, considerando esta espécie também como potencial fornecedora de cortiça para a indústria corticeira. A proporção de cortiça na casca aumenta com a idade da árvore e torna-se interessante em termos de rendimento para árvores maduras com mais de 35 anos de idade cambial. A integração da valorização da casca desta espécie aquando da exploração madeireira atual para as serrações aconselha o aproveitamento da casca na parte inferior dos troncos das árvores; nestes toros poderá proceder-se ao descasque para aproveitamento da casca, fraccionamento da sua cortiça e posterior aproveitamento.

A estrutura da cortiça presente na casca desta espécie foi estudada, avaliando-se as suas características em termos de arranjo topológico, geometria e dimensões das células e fazendo uma discussão tendo em conta o impacto nas propriedades e potencial utilização como produto. Este trabalho permitiu verificar que, no ritidoma da casca desta espécie a cortiça se encontra intercalada com floema formando uma estrutura descontínua e heterogénea. Em termos celulares foi verificada a existência de zonas onde as células de cortiça se encontram esmagadas e completamente colapsadas, formando uma estrutura compacta entre zonas com células descompactadas. Nas regiões de células não colapsadas, a maioria das células de cortiça apresenta-se sob a forma de prismas com, em média, 55 µm de altura e 1388 μm^2 de área da base e 1,3 µm de espessura de parede celular. Esta cortiça possui características semelhantes às das células de cortiça de *Quercus suber*, embora a sua utilização só seja possível após um processo prévio de expansão celular das zonas colapsadas. A utilização desta cortiça

como material irá requerer um processo de trituração e fragmentação para a obtenção de fracções puras de cortiça de casca de pseudotsuga que poderá depois ser utilizada sob a forma de granulado para aglomerados.

Fez-se também o estudo das características químicas da casca de pseudotsuga e a sua variação com a idade cambial. Verificou-se que a composição química da casca varia consoante a idade, especialmente ao nível da quantidade de extrativos, lenhina e, principalmente, suberina. Com 45, 30 e 17 anos de idade, a casca contem respectivamente 25,4 %, 2,6 % e 0,9 % de suberina, 24,5 %, 33,9 % e 29,8 % de lenhina, e 29,4 %, 20,6 % e 25,7 % de extractivos. As diferenças químicas são consequência da variação estrutural com a idade, principalmente do número de peridermes e do teor de cortiça que é pequeno em cascas com menos de 30 anos. Deste modo uma utilização da casca dirigida ao aproveitamento da cortiça deverá apenas considerar a parte inferior dos troncos de árvores maduras. Também se analisaram as diferenças químicas entre cortiça e floema que diferem em relação a teor de extractives (49,8 % vs. 17,0 %), suberina (30,1 % na cortiça vs. não presente no floema) e composição de hemiceluloses em relação ao teor de arabinose (25,3 % vs. 4,8 % dos monosacáridos respetivamente). A composição monomérica da suberina mostrou diferença entre a cortiça proveniente dos dois locais relativamente à proporção de monoácidos e ω-hidroxiácidos: a suberina é constituída maioritariamente por diácidos alcanóicos (45,0 % e 46,1 % dos monómeros alifáticos identificados) e também por ácidos alcanóicos (26,2 % e 16,8 %) e ω-hidroxiácidos (26,3 % e 34,0 %).

A análise dos extractivos polares das cascas permitiu verificar que são ricos em fenóis com alto poder antioxidante, o que constitui uma via de valorização da casca total.

Os resultados obtidos com este trabalho constituem informação importante, quer a nível nacional quer internacional, para a caracterização da casca e cortiça de *Pseudotsuga menziesii*. A exploração dos povoamentos existentes desta espécie bem como a sua potencial expansão dado o grande potencial desta espécie para a floresta portuguesa para fins madeireiros, poderá incluir o aproveitamento da sua casca com vista a uma maior valorização dos produtos florestais não lenhosos.

Palavras-chave: Pseudotsuga; Casca; Cortiça; Anatomia; Química

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Introduction and Objectives

1. Introduction

Douglas-fir is one of the most important commercial coniferous timber tree species in the world (Kleinschmit and Bastien, 1992; Forst et al., 2010; Lavender and Hermann, 2014) with notable characteristics as rapid growth and high quality timber (Hermann and Lavender, 1999; Savill and Wise, 2013).

Native from the Western North America region, it was introduced in Europe and the interest in Douglas-fir improvement has grown in conjunction with the growing use of the species in European forestry.

In Portugal, the species was introduced in the 19th century and in general spread as individual trees planted in parks and gardens, and small plantations established by the Portuguese Forest Service (Carvalho, 1965). The high potential for this species in the mountain areas in the center and north of Portugal has been recognized due to its fast growth, ability to grow under a wide variety of conditions, natural regeneration, adaptability and a high potential for timber production (Diniz, 1969; Goes, 1976; Woods, 1976; Fontes, 1989; Louro and Cabrita, 1989; Goes, 1991). The area of Douglas-fir in Portugal is 4,200 ha (Martins, 1999) and represents only about 0.1 % of the country's total forest cover (Fontes, 2002; Lavender and Hermann, 2014). Nevertheless there is an estimated area of 250,000 ha where Douglas-fir trees could be planted and will exceed 17 m dominant height at 30 years corresponding to 8 % of the existing Portuguese forest area. It has the potential to be an excellent option for the Portuguese Forestry Sector, and could become the fifth most common forest tree species after maritime pine, cork oak, eucalyptus and holm oak (Fontes, 2002).

The bark of trees, a non-wood forest product, may supply different kinds of products like cork, tannin, dyes, and others, with different applications. The main utilization possibilities of bark are energy (e.g. incineration), composting, materials (e.g. cork, fibers, composites), chemicals (e.g. extractives, bio-oils) and absorption (Roth, 1981). Nevertheless barks are among the most abundant and under-utilized residual bioresources, usually considered as a waste product from forest operations and forest industrial processes (Lu et al., 2016). In the biorefineries concept, e.g. the use of biomass

for chemicals, materials and energy (Tuck et al., 2012; Le Normand et al., 2014), bark offers many possibilities because of its complex chemical composition.

The bark utilization of specific forest species implies knowledge of their properties, namely their anatomy, chemistry and physics. These studies are essential to determine a technological application and appropriate uses of the bark of the species (Rios, 2007).

One of the valuable fractions of the bark is the cork and there are a number of tree and shrub species where the cork attains exceptional thickness, eventually building the so-called ‘‘corky barks’’. Various studies indicate that there are several tree species that contain cork tissues in their barks like *Betula pendula* (Pinto et al., 2009), *Quercus cerris* (Şen et al., 2011a, 2011b), *Quercus variabilis* (Chinese cork oak) (Miranda et al., 2013b; Ferreira et al., 2016a), *Kielmeyera coriacea* Mart. and Zucc (Rios, 2011) as well as Douglas-fir (*Pseudotsuga menziesii*) (Hergert and Kurth, 1952; Grillos, 1956; Hall, 1971; Ross and Krahmer, 1971; Krahmer and Wellons, 1973; Litvay, 1973, 1976; Patel, 1975; Laver et al., 1977; Litvay and Krahmer, 1977; Dougal, 1981; Marques et al., 2006). A few other tree barks have also been considered and studied as potential sources of cork.

The most exceptional case is the cork-oak tree (*Quercus suber* L.), that grows in the western Mediterranean region and from which cork is obtained and industrially processed, with most of the world production coming from Portugal and Spain (Pereira, 2007). In Portugal there are more than 720 thousand hectares of cork forests, and a cork industry of considerable economic importance (APCOR, 2015). Cork has unique and incomparable qualities: it is very light, impermeable to liquids and gases, elastic and compressible, an excellent thermal and acoustic insulator, fire protector and highly abrasion resistant (APCOR, 2015). Cork is an important non-wood forest product that has a wide range of uses, including wine and champagne stoppers, insulation, floats for fishing net and bulletin boards (Ciesla, 2002).

Since Douglas-fir bark has a high content of cork, and since it is very abundant, researchers proposed methods for removing the cork (Grillos, 1956). Some parts of the Douglas-fir bark, particularly from the bottom of mature trees, contain an excess of 50 % by weight of cork (Hergert and Kurth, 1952) or an average around 33 % of cork (Hall, 1971). The cork content of the bark varies considerably, depending upon the age

of the tree and the position of the bark on the tree but deep cork formation usually occurs at a relatively early age (Hergert and Kurth, 1952).

Since Douglas-fir is one of the best timber conifers in the world with high value wood and has the potential to be an excellent option for the Portuguese forestry (Fontes, 2002), this work contributes for the full valorization of this species. Given the importance of the cork industry in Portugal, it appears useful to have more knowledge on this bark development and characteristics, namely on its cork component.

With this study we wish to contribute to the valorization of the bark of Douglas-fir through its anatomical and chemical characterization and evaluation of its development in comparison with the properties of the cork of *Quercus suber* that is the reference material.

2. Objectives and overview

The research carried out in this work aimed to bring new knowledge on the development of Douglas-fir bark on trees grown in Portugal and to contribute for the potential utilization of these bark residues through the valorization of its cork component.

The specific objectives were:

1. To study the within-tree age-related variation of the stem components heartwood, sapwood and bark of Douglas-fir;

2. To analyse if the bark of Douglas-fir can be considered as a source of cork and at what age and/or height level this will be possible based on bark structural variation and development;

3. Anatomical and chemical characterization of the cork fraction of Douglas-fir bark and its within-tree age variation in view of its application.

This Phd thesis is structured in four chapters and the results are presented as internationally refereed papers published or submitted in scientific journals of the corresponding specific areas.

This first chapter, named Introduction and objectives, presents a brief introduction of the thesis, the main objectives and the list of publications.

The second chapter, entitled State of the art, presents the state of the art related to the subjects dealt with in this work. It gives a general description of the *Pseudotsuga menziesii* tree, the importance of bark, the formation of bark and includes the existing studies on pinaceae barks and on the barks with cork, and finally the anatomical and chemical characterization of cork.

The third chapter, named Original research, presents the original research of this work and starts with an outline, followed by the material and methods used, and afterwards the results organized by the publications: two articles published and two articles submitted in international journals with referee.

The fourth chapter includes the conclusions and the perspective of future works.

3. List of publications and presentations

Publications:

I. Cardoso, S. and Pereira, H. (2017) Characterization of Douglas-fir grown in Portugal: heartwood, sapwood, bark, ring width and taper. European Journal of Forest Research, 136 (4): 597-607. DOI: 10.1007/s10342-017-1058-z

II. Cardoso, S., Ferreira, J., Quilhó, T. and Pereira, H. (2017) Cork of Douglas-fir bark: impact of structural and anatomical features on usage. Industrial Crops and Products, 99: 135-141. DOI: 10.1016/j.indcrop.2017.02.001

III. Cardoso, S., Quilhó, T. and Pereira, H. (2019) Influence of cambial age on the bark structure of Douglas-fir. Wood Science and Technology, 53 (1): 191-210. DOI: https://doi.org/10.1007/s00226-018-1055-5

IV. Cardoso, S., Ferreira, J., Miranda, I. and Pereira, H. (2018) Age variation of Douglas-fir bark chemical composition. Journal of Wood Chemistry and Technology, 38 (5): 385-396. DOI: 10.1080/02773813.2018.1513036

Presentations:

I. Cardoso, S., Miranda, I., Pereira, H. (2017) Age-related chemical variation of *Pseudotsuga menziesii*: bark. In: 2nd ULisboa Chemistry PhD Meeting, Lisboa, Portugal, 4-5 Dec

II. Cardoso, S., Quilhó, T., Pereira, H. (2017) *Pseudotsuga menziesii*: uma fonte potencial de cortiça. In: 8º Congresso Florestal Nacional – Floresta em Português: Raízes do Futuro, Viana do Castelo, Portugal, 11-14 Oct

III. Cardoso, S. and Pereira, H. (2017) *Pseudotsuga menziesii*, an alien tree species, is a potential supplier of cork. In: National Science Summit'17, Centro de Congressos de Lisboa, Portugal, 3-5 Jul

IV. Cardoso, S. and Pereira, H. (2017) Douglas-fir (*Pseudotsuga menziesii*), an alien tree species, is a potential supplier of cork. In: 4th Annual Conference (Interdisciplinary network on agro-food and forestry) – Foster Innovation through Resilient and Efficient Agro Food & Forestry Systems. 3rd ULisboa Innovation Week. Lisboa, Portugal, 3-9 May

State of the art

1. Douglas-fir

1.1. Taxonomy and distribution

Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) was discovered by the Scottish explorer Archibald Menzies, in 1792, on the west coast of Vancouver Island, British Columbia, at Nootka Sound. The genus *Pseudotsuga* belongs to the subfamily *Abietodeae* of the family Pinaceae. The type species is *Pseudotsuga menziesii* (Mirb.) Franco. Douglas-fir was placed consecutively in the genera *Pinus*, *Abies*, *Picea* and *Tsuga* until Carrière established a separate genus, *Pseudotsuga* (Hermann, 1982). In the 19th century, European settlers and lumbermen in the American West coined numerous names for Douglas-fir e.g. red fir, yellow fir, Douglas spruce, cork-barked Douglas spruce, Oregon pine, red pine, red spruce, fir, spruce, Douglas yellow fir, black fir, etc. The name Douglas-fir was adopted by the U.S. Forest Service after a census of lumbermen revealed that it was more used than all the other names combined (Hermann, 1982).

Pseudotsuga menziesii (Mirb.) Franco var. *menziesii* is the coastal variety of Douglas-fir that occurs in a broad coastal band which lies to the west of the coastal range in British Columbia, the Cascade Range in Washington and Oregon, and the Sierra Nevada in northern California (Fontes, 2002) (Figure 1). Douglas-fir is native to North America where it has a wide distribution, occupying approximately 19 million ha in the USA and Canada (Weiskittel et al., 2012). The species was introduced outside its natural range in various regions, namely in Europe nearly 200 years ago, where it is now the most widely distributed North American conifer occupying over 550 thousand ha (Lavender and Hermann, 2014). Douglas-fir became a major reforestation species in Western Europe after the Second World War, mainly with the support of national or regional forest grants (Pâques, 2013). At present, the countries with the largest share of Douglas-fir plantations in Europe are France, Germany, the United Kingdom and the Netherlands (Lavender and Hermann, 2014). Interest in Douglas-fir improvement has grown in conjunction with the increasing use of Douglas-fir in European forestry.

Figure 1 - Natural distribution of the Douglas-fir (Little, Jr., 2018)

In Portugal, the species was introduced between 1844 and 1849 through individual tree plantings in Sintra (Figure 2) (Gomes and Raposo, 1939; Lavender and Hermann, 2014). The first Douglas-fir forest plantations were established by the Forest Service in Serra da Estrela, in 1904 (Freitas, 1989), and in Serra do Gerês, in 1906 (Coutinho, 1936). In general, Douglas-fir was little noticed and used until the last quarter of the 20th century, except for individual trees planted in parks and gardens, and small plantations established by the Portuguese Forest Service (Carvalho, 1965). Douglas-fir occurs mainly in the North (Serras de Bornes, Padrela, Marão and Gerês) and Center (Serra da Estrela), particularly in Penhas Douradas and Manteigas (Marques et al., 2008). The seed origin used in the Portuguese plantations is unknown (Diniz, 1969; Goes, 1976; Woods, 1976; Louro and Cabrita, 1989), and no information on the best provenances, in terms of adaptability and growth, is available (Fontes, 2002).

Figure 2 - First Douglas-fir tree planted in Portugal at Sintra

The area of Douglas-fir in Portugal is 4,200 ha (Martins, 1999) and it represents only about 0.1 % of the country's total forest area (Fontes, 2002; Lavender and Hermann, 2014). Nevertheless there is an estimated area of 250,000 ha where Douglas-fir trees could be planted and will exceed 17 m dominant height at the age of 30 years corresponding to 8 % of the existing Portuguese forest area (Fontes, 2002) (Figure 3). In fact the high potential for this species in the mountain areas, in the center and north of Portugal, at 700 to 1,000 m elevation, and with a moisture deficit above 400 mm has been recognized due to its fast growth, ability to grow under a wide variety of conditions, natural regeneration, adaptability and a high potential for timber production (Diniz, 1969; Woods, 1976; Goes, 1976, 1991; Fontes, 1989, 2002; Louro and Cabrita, 1989).

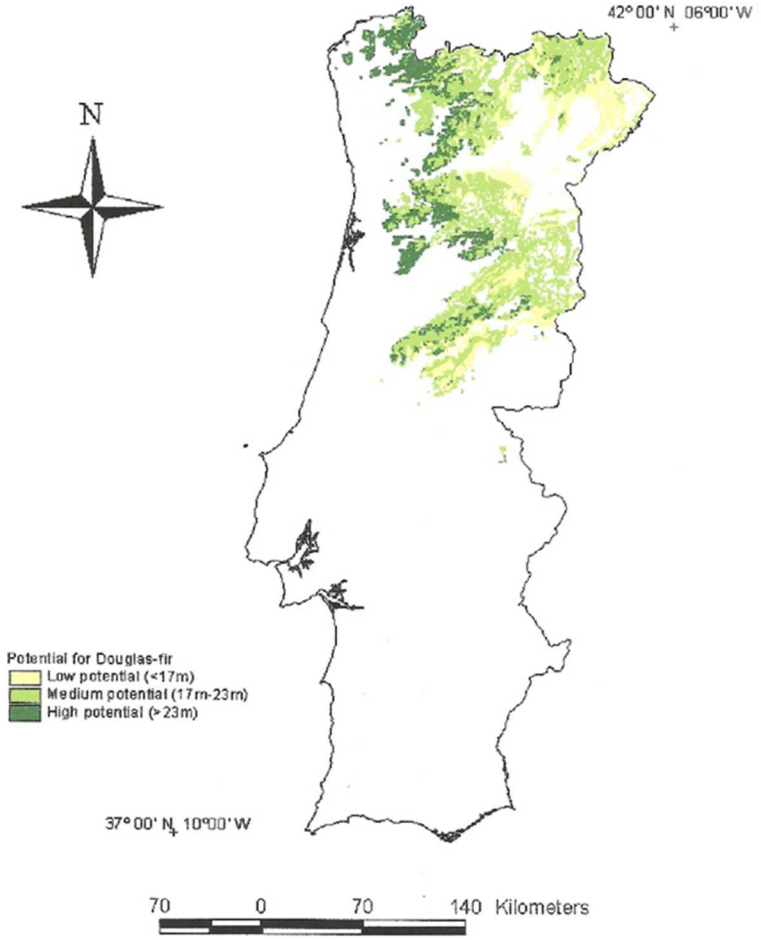

Figure 3 - Estimated potential area of distribution for Douglas-fir production (Fontes, 2002)

Douglas-fir in Portugal performed better in terms of height growth than several other species, like *Pinus pinaster* Aiton, *Castanea sativa*, *Pinus nigra* var. *maritima* (Ait.) Melv., *Cedrus atlantica* (Endl.) Carr. and *Pinus sylvestris* L. (Maia et al., 1990). As said by Hernandez et al. (1993): "The Pacific and interior valley climates of western Washington, western Oregon, and northern California match those of the Iberian Peninsula so closely that Douglas-fir may have greater potential in Spain and Portugal than in the rest of Western Europe." The species has the potential to be an excellent option for the Portuguese forestry, and could become the fifth most common forest tree species after maritime pine, cork oak, eucalyptus and holm oak (Fontes, 2002).

1.2. Description and silviculture

Douglas-fir (Figure 4) is the largest tree in North America after the giant sequoias of California (Harlow et al., 1979). It is a tree up to 100 m height and 4 m in diameter that may attain a great age, over 700 years (Vidakovic, 1991).

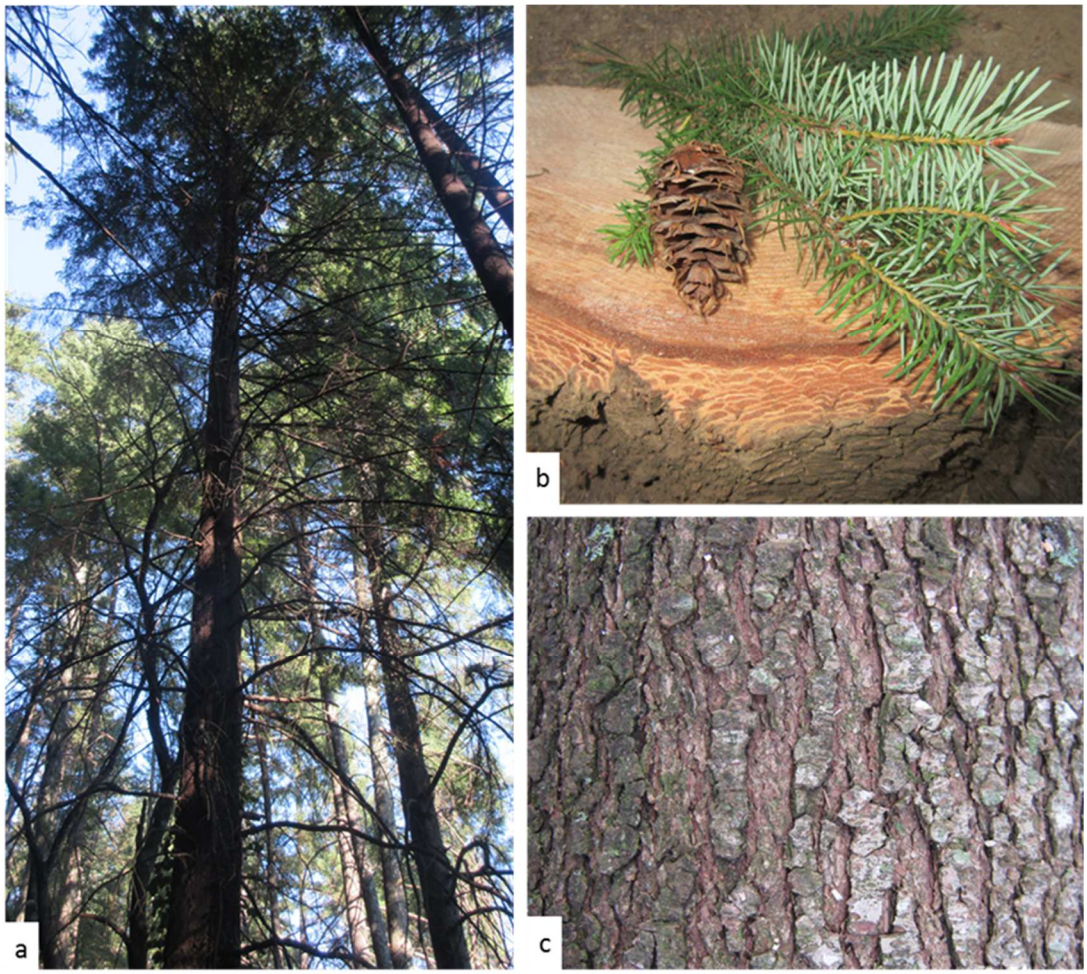

Figure 4 - Douglas-fir tree appearance (a), wood, needles, cones (b) and bark (c)

The crown of young trees is conical with ascending branches, while older trees have broader crowns. The branches are horizontal, with the lower ones somewhat dropping. The needles are straight, 2-3 cm long, light green to dark green, flat or partly so, arranged around the shoot, with a blunt or pointed apex, narrow at the base. The flowers appear in April and May. The cones are cylindrical, 6.5-10 cm long and 3-3.5 cm across. The seed is about 6 mm long, glossy, dark brown, mottled below, ripening by the end of August and beginning of September. The bark of young trees is smooth with many

resin blisters, and on old trees bark becomes reddish-brown, thick, corky, and deeply fissured (Vidakovic, 1991).

Douglas-fir is characterized by rapid growth, longevity and several characteristics that have evolved to take advantage of sites with high or moderate fire severity regimes. These include the thick, corky and fire-resistant bark on the lower bole, thereby protecting the cambial regions from the heat of fires. The wind-dispersed seeds facilitate long distance dispersal to disturbed sites (Pyne, 1984; Pyne et al., 1996).

In Europe, Douglas-fir is one of the fastest growing trees, growing on a wide range of soils, but performing best at mid-elevation (up to 900 m) with an annual rainfall over 800 mm. On good sites, maximum heights can reach 30 m at 40 years of age. Depending on the site, volume production can be between 600 and 800 m^3 at age 40 (i.e. mean annual increment of 15-20 m^3 per ha and year) (Pâques, 2013).

Douglas-fir does not tolerate serious exposure to wind and is usually regarded as a species best suited to the middle and lower slopes of valleys with at least moderate fertility. It needs a deep, well-drained soil in order to develop a good root system; the soil can be clayey, if on a slope, and the tree will grow well on sandy soils but not on alkaline soils (Pâques, 2013; Savill and Wise, 2013). In plantations, Douglas-fir is not suited to grow mixed with other species, especially broadleaved species, because of its rapid growth that tends to suppress them (Savill and Wise, 2013).

Douglas-fir trees are usually harvested after they are 40 years old, and rotation lengths greater than 70 years are possible (Howe et al., 2006). The most common rotations in Portugal are between 40 and 70 years and it is generally cultivated in monoculture (Fontes, 2002). However, plots of Douglas-fir mixed with *Castanea sativa* have shown promising results in terms of productivity in Northern Portugal (Luis and Monteiro, 1998; Nunes et al., 2014). After forest fires, Douglas-fir grows naturally in vast, almost pure, stands as a result of its rapid establishment.

Natural pruning in Douglas-fir plantations is very slow and since the quality and the value of knot-free wood is significantly higher, the pruning of selected trees is recommended. Knots in the wood compromise its use for furniture or veneer and create more difficulties for sawing. However, pruning is not a common practice in Portugal.

In Portugal, Douglas-fir wood is ranked low in density compared with wood from its native distribution and microfissuration can occur. The occurrence of microfissures does not significantly reduce the quality as it is usually limited to the juvenile core and practices to minimize microfissuration can be adopted such as close initial spacing to reduce the core of juvenile wood and avoiding areas with unsuitable site and climatic conditions (Fontes, 2002).

1.3. Applications and economic value

Douglas-fir is one of the world premier coniferous timber trees and also an important economic species in Europe because of its characteristics of rapid growth, high quality timber, high reproduction capacity, adaptation, and low number of pests and diseases (Kleinschmit and Bastien, 1992; Miller and Knowles, 1994; Hermann and Lavender, 1999; Savill and Wise, 2013; Da Ronch et al., 2016).

Douglas-fir has gained a wide acceptance as one of the finest softwoods and is probably one of the best known softwoods (Bishop, 1999; Walker et al., 2013). Since the trees may reach between 40 to 60 m of height, of which two-thirds will be a clear, slightly tapering bole up to 2.4 m in diameter, significant amounts of clear grade lumber can be produced (Bishop, 1999; Walker et al., 2013). Douglas-fir stands are very productive over longer periods and its timber has excellent strength properties, stability in use, and moderate durability out of contact with the ground (Forst et al., 2010; Walker et al., 2013).

The sapwood is usually not wider than about 5 cm and is slightly lighter in colour than the heartwood. The heartwood has clearly defined early and latewood growth rings, producing a distinct wavy pattern on tangential faces. The wood ranges from a pale to dark brown over these growth rings. Some resin ducts and pockets are present. This medium-weight softwood is rated as much stronger than *Pinus sylvestris* and more resistant to bending, and it has good resistance to wear nearly up to that of *Pinus palustris* and *Pinus elliottii* (Bishop, 1999). It also dries with little degrade (Savill and Wise, 2013). Douglas-fir wood works reasonably well with sharp tools although with a tendency to split, it glues well and can be polished to a satisfactory finish (Bishop, 1999).

Knot-free Douglas-fir is valued for veneering, top quality joinery, construction, structural work and decoration (Bishop, 1999; Savill and Wise, 2013; Walker et al., 2013). It can be peeled for use in exterior grade plywood and engineered products (e.g. laminated veneer lumber) (Bishop, 1999; Howe et al., 2006).

Douglas-fir timber attracts higher prices than most other conifers. Markets for small sizes are similar to those of spruce and pine, and for large timber (60-90 cm d.b.h.) there is a distinct market for knot-free structural beams for major building projects (Savill and Wise, 2013).

In Portugal, Douglas-fir wood is used for construction, medium-density fibreboard (MDF) and particleboards (Carvalho, 1997). The occurrence of knots may hinder its use for furniture (Carvalho, 1965). Due to the small area that Douglas-fir represents in Portugal, there is not much information about its markets. However, in 2018, it was sold for about 26 €/m^3 and 36 €/m^3 for 25-30 cm d.b.h. and larger than 30 cm, respectively, whilst *Pinus* spp sold for 21 €/m^3 and 26 €/m^3 (ICNF, 2018)

The potential use of Douglas-fir bark as a raw material was already analyzed in early papers published since the 50's of last century. Research on Douglas-fir bark began in 1942, when the Development Department of the Weyerhaeuser Timber Company began experiments with cork with the idea to find a suitable cork material to supplement the decreasing cork supply from the Mediterranean during World War II (Percival, 1948). During the research in separating cork particles from bark, other parts in the bark offered greater promise as industrial raw material and a study was published on the Douglas-fir bark structure regarding its cork and tannin utilization (Percival, 1948). The chemicals of Douglas-fir bark were studied focusing on tannins (Hubbard and Kurth, 1949), wax (Kurth, 1950; Kurth and Kiefer, 1950) and chemical nature of cork (Hergert and Kurth, 1952), also giving a brief description of the microscopic structure of bark (Kurth, 1953).

In 1958, Hobart and Murray patented the separation of cork from the Douglas-fir trees and subsequent results of application for floor tiles were presented as well as the production of particleboard with Douglas-fir bark without additives (Burrows, 1959, 1960).

Holmes (1961) studied the chemical composition of extractives and identified some of the relatively simple compounds in the newly formed inner bark extract. Hall (1971) reviewed the utilization of the bark of Douglas-fir and presented different applications that included fuel, pyrolysis and charcoal manufacture, board and tile manufacture, agricultural use as well as utilizations of different fractions: cork, fiber, powder and whole-bark extracts. Chen (1973) reported the isolation and characterization of a holocellulose fraction from Douglas-fir bark. Laver et al. (1977) reviewed the research since 1971 on the chemical constituents of Douglas-fir bark and emphasized wax, carbohydrates, cork, and condensed tannins. Aslam et al. (1989) studied the production of oxalic acid by catalytic oxidation of Douglas-fir bark and subsequent pyrolysis of the residue to produce high density carbon pellets.

2. The bark of trees

2.1. Definition and functions

The trees have developed an often rugged outer skin referred to commonly as bark that covers externally branches and stem (Morris and Jansen, 2016). Bark represents 9-15 % of the stem volume (Harkin and Rowe, 1971). Bark varies considerably from species to species in thickness, texture and color (Figure 5) and within a species also with tree age, rate of growth, genotype and location (Cunningham, 2001; Leite and Pereira, 2017). Junikka (1994) standardized for the first time the terms to describe the macroscopic features of bark (e.g. texture, patterns and exudates) bringing more clarity in a rather confusing bark terminology.

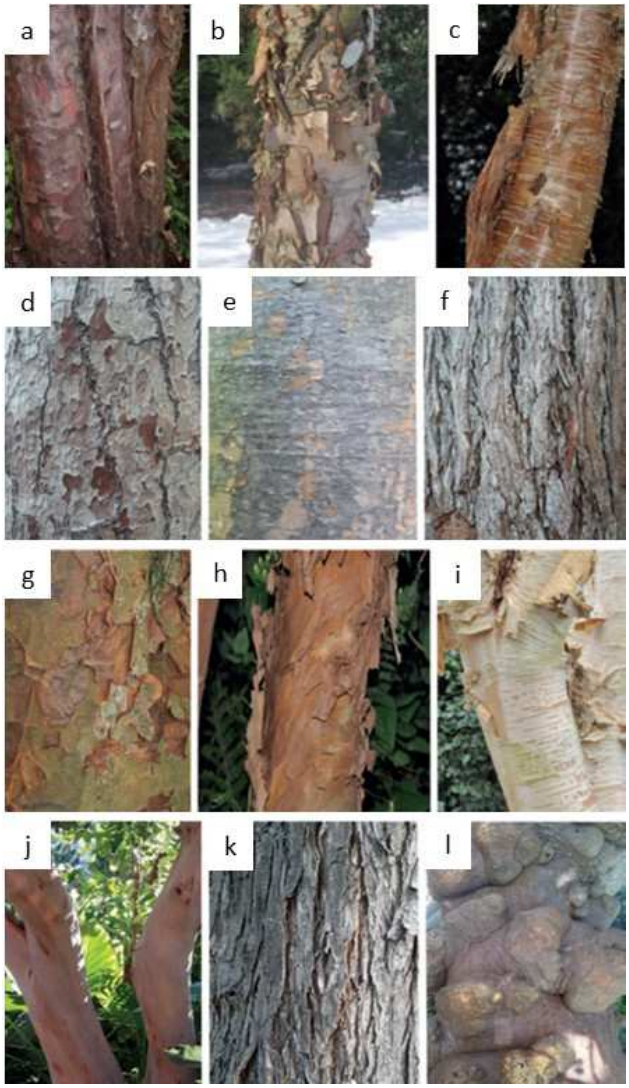

Figure 5 - The diversity of barks a) *Cupressus guadalupenis*, b) *Betula nigra*, c) *Prunus maackii*, d) *Pinus canariensis*, e) *Agathis dammara*, f) *Eucalyptus robusta*, g) *Acer griseum*, h) *Fuchsia exocorticata*, i) *Betula utilis*, j) *Arbutus canariensis* k) *Schinus terebinthifolius* l) *Erythrina americana* (Morris and Jansen, 2016)

The bark is multifunctional, but the different functions may be more or less prominent depending on the environment in which the species grows. The key functions of bark include: protection, aeration, water storage, photosynthesis and mechanical purpose (Lev-Yadun, 2011; Morris and Jansen, 2016). A selective pressure exerted on bark from the environment determines its appearance, overall thickness and the thickness ratio between outer and inner bark. An ecologically important function of bark is to protect trees from fires (Gill and Ashton, 1968; Vines, 1968; Roth, 1981; Paine et al., 2010; Pausas, 2015). Thick barks, being poor conductors of heat, isolate the sensitive living tissues of many tree species from fires (Lev-Yadun, 2011) and a thick outer bark may be key to protection of inner tissues from fire or large vertebrates, such as mammals (Morris and Jansen, 2016).

The bark includes all tissues outside the vascular cambium e.g. primary and secondary phloem, first periderm and sequent periderms (rhytidome) (Esau, 1953, 1977; Fahn, 1990; Evert, 2006) (Figure 6).

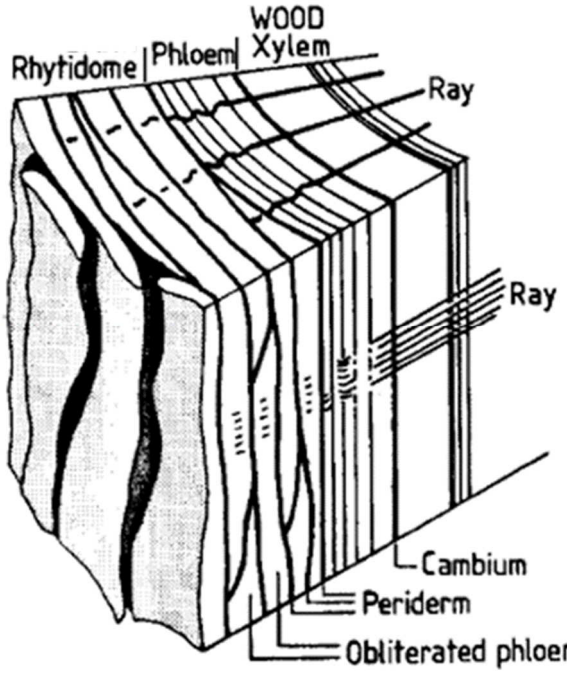

Figure 6 - Schematic representation of the bark and the various tissues that compose it (Fengel and Wegener, 1983)

However, the nomenclature and terminology of bark was not consensual until recently. Some authors considered the bark only as the periderm and rhytidome (Chattaway,

1955a, 1955b, 1955c); others included the phloem in the bark considering the terms of outer bark and inner bark assigned respectively to the rhytidome and to the secondary phloem (Howard, 1971; Bramhall et al., 1977; Furuno, 1990); the terms inner and outer bark were also used for the functional and non-functional phloem (Schaad and Wilson, 1970), also referred to as conductive and non-conductive phloem (Nanko et al., 1979; Chan, 1985, 1986; Trockenbrodt, 1990; Donghua and Xinzeng, 1993). Trockenbrodt (1990) considers as the main criterio for distinguishing between these two phloem zones the collapse of the sieve tube elements, and designated these zones by non-collapsed phloem and collapsed phloem.

A lack of a consistent and internationally accepted list of bark anatomical features contributed to a difficult description and analysis of bark (Crivellaro and Schweingruber, 2013). Trockenbrodt (1990) and Lev-Yadun (1991) pioneered the attempt to establish some criteria for the description of the bark and Richter et al. (1996) published for the first time a glossary for bark. Recently, Angyalossy et al. (2016) published the IAWA List of microscopy bark features, thereby establishing a terminology like the one that exists for wood.

Bark anatomy is still rarely presented systematically for many species although the bark potential utilization of specific species implies knowledge of their properties, namely their anatomy, chemistry and physics (Angyalossy et al., 2016).

The use of barks was recognized since ancient times (Lev-Yadun, 2011). Their richness in chemical compounds as well as their special physical properties (Roth, 1981) allow different kind of uses:

- for food (Nishida, 1976; Sandved et al., 1993; Östlund et al., 2009);
- in medicine (Turner and Hebda, 1990; Sandved et al., 1993; Lee and Ahn, 1998; Shah et al., 1998; Jham et al., 2005; Kilani, 2006);
- in household e.g. condiments, detergents (Shah et al., 1998; Jham et al., 2005);
- for energy e.g. fuel, charcoal (Yamato et al., 2006; Rhén et al., 2007; Frankó et al., 2015);
- in agriculture e.g. fertilizer or mulch, potting medium (Yamato et al., 2006);
- as wood substitute e.g. insulation boards, fiberboards, particleboard (Chow, 1979; Roffael et al., 2000; Xing et al., 2006);

- in air and water pollution (Roth, 1981; Van Langenhove et al., 1986; Brás et al., 1999);
- for cork products (Leite and Pereira, 2017);
- as fibers e.g. plaited mat-works or wicker goods, wrapping, paper (Chow, 1979; Yemele et al., 2010);
- as a source of tannins (Roffael et al., 2000), rubber e.g. latex (Roth, 1981; Yeang, 1988; Kongsawadworakul et al., 2009) and extractives for e.g. adhesives, binders, antioxidants, cosmetics, fungicides, bactericides (Chow, 1979; Roth, 1981; Lee and Ahn, 1998; Chang et al., 2001; Kilani, 2006).

The roundwood world production was about 3,591 million m^3 in 2013 (FAO, 2015), generating over 300 million m^3 of bark that are largely concentrated at processing sites and industrial mills. Bark is still considered as a residue and known to be either left in the forest after tree felling or used as a fuel by the forest industry (Lu et al., 2006). However the barks obtained as residues in forestry and primary wood processing industry are also a valuable raw-material for biorefineries due to their structural and chemical diversity (Harkin and Rowe, 1971; Le Normand et al., 2014).

2.2. Formation and anatomy

The formation of the bark in the tree is the result of the activity of the vascular cambium and of the phellogen, as described in plant anatomy reference books (Esau, 1977; Fahn, 1990; Evert, 2006).

The vascular cambium that encircles circumferentially the stem gives rise to the xylem (wood) inwards and to the secondary phloem outwards, by periclinal cell divisions; the phellogen, or cork cambium, that usually is not continuous around the stem, differentiates cells by periclinal divisions and forms outwards the phellem and inwards the phelloderm; the phellogen, phellem and phelloderm make up the periderm. To allow for diameter increment, the vascular cambium and the phellogen cells also perform occasional anticlinal divisions, thereby increasing the number of radial rows.

When the external tissue of the periderm is no longer able to accommodate radial growth, it cracks, dies and is replaced by a new functioning periderm, each time forming deeper inside the living phloem tissues. Consequently, bark accumulates to the outside of the functioning periderm layers of dead nonfunctional periderms and phloem tissues between them, forming the so-called rhytidome. So the bark is not homogeneous and contains, from inside to outside, different tissues corresponding to the secondary phloem, periderm and rhytidome.

The secondary phloem is composed of conducting and nonconducting phloem (Angyalossy et al., 2016). The innermost portion of the phloem located near the vascular cambium is the functioning phloem, where sieve elements conduct photosynthates; the nonconducting phloem represent the part of the phloem that has lost its conducting capacity by the crushing of sieve elements and a disarray of tissues.

The phloem has different types of cells such as the sieve elements (sieve cells or sieve tube elements in softwoods and hardwoods respectively) for water and organic material transfer, the axial and the radial parenchyma for storage and transport, the fibers that provide mechanical support and secretory cells. In the non-conducting phloem, a dilatation tissue is commonly observed to adjust the secondary phloem to the radial tree growth i.e. proliferation and enlargement of axial or radial parenchyma cells, formation of sclereids and dilated rays. The proportion of cell types and their arrangement differ between species and in a single species (Trockenbrodt, 1994; Quilhó et al., 1999, 2000; Gricar et al., 2015).

The periderm is a secondary protective tissue developed by the activity of the phellogen that replaces the epidermis in stem and roots. Initiation of the first phellogen occurs at different ages and sites depending on species, and the site of initiation may vary within the same family and even within the same plant (Chiang, 1978; Leu and Chiang, 1981). The subepidermal cortex layer is the most common site of origin of the first periderm (Angyalossy et al., 2016) but in some cases it appears in the epidermis or in the phloem (Evert, 2006; Pereira, 2007). The phellogen activity, like that of the vascular cambium, is seasonal with periods of dormancy and of activity depending on environmental conditions, namely light, water and temperature. Duration of the first phellogen is quite different between species but in general has a limited lifespan; an exception is the cork

oak (*Quercus suber*) in which the first phellogen is active throughout the entire life of the plant (Natividade, 1950; Pereira, 2007).

The phellem or cork tissue is fairly homogeneous with a regular arrangement of cells, usually tightly packed and characterized by a cell wall containing suberin that is internally deposited onto the primary cell wall, forming a layer to prevent water loss and provide protection against temperature variation, fire and biological attack (Graça and Pereira, 2004; Pereira, 2015). The cork cells anatomical and chemical structure will be described in a following item. Unsuberized cells can also occur in the phellem, usually called phelloids (Coder, 2014; Quilhó et al., 1999): e.g. thin-walled suberized cork cells alternate with bands of thick-walled, unsuberized, stone cells in pines (Howard, 1971), in *Pinus pinea* and *Pinus pinaster* (Nunes et al., 1996, 1999); and unsuberized cells occur in the phellem of *Eucalyptus globulus* (Quilhó et al., 1999).

The longevity and activity of the phellogen are decisive factors to determine the thickness and homogeneity of the cork tissue; therefore the phellem ranges from thick to thin, corky to non-corky, and the number of cork layers is very variable between species and with plant age, and may be very large as in the cork oak (Pereira, 2007; Leite and Pereira, 2017).

The phelloderm cells are living cells with non-suberized walls that resemble parenchyma cells but are identified by their arrangement in radial rows under the phellogen initials. In general, the phellogen is less active on the side of phelloderm, i.e. less phelloderm is formed comparatively to the phellem (Dickison, 2000).The number of phelloderm layers differs depending on the tree species (Evert, 2006) but, in some cases, tropical trees seem to have a very thin phellem layer but thick phelloderm (Roth, 1981).

The rhytidome consists of the innermost periderm and of tissues isolated by it to the outside, namely the more peripheral periderms, phloem tissues, and cortical tissues and epidermis until they are shed. The rhytidome formation occurs by the successive development of periderms, and barks that have only one superficial periderm by definition do not form a rhytidome (Roth, 1981; Evert, 2006). Therefore, bark consists of phloem and rhytidome, and its macroscopic appearance and properties will depend

on the structure of these tissues, their extent, and relative proportion (Huang et al., 2006).

The surface morphology of the bark derives from the structure of the rhytidome (number of periderms and their cellular features and development) and the cellular composition and arrangement of the phloem tissues (e.g. proportion and arrangement of fibers), often giving the unique bark features of a particular species, like depth and direction of wrinkling and kind of exfoliation (Roth, 1981; Beck, 2010).

Figure 7 represents a schematic diagram of a portion of the rhytidome of a typical southern pine showing the respective layers and cellular elements.

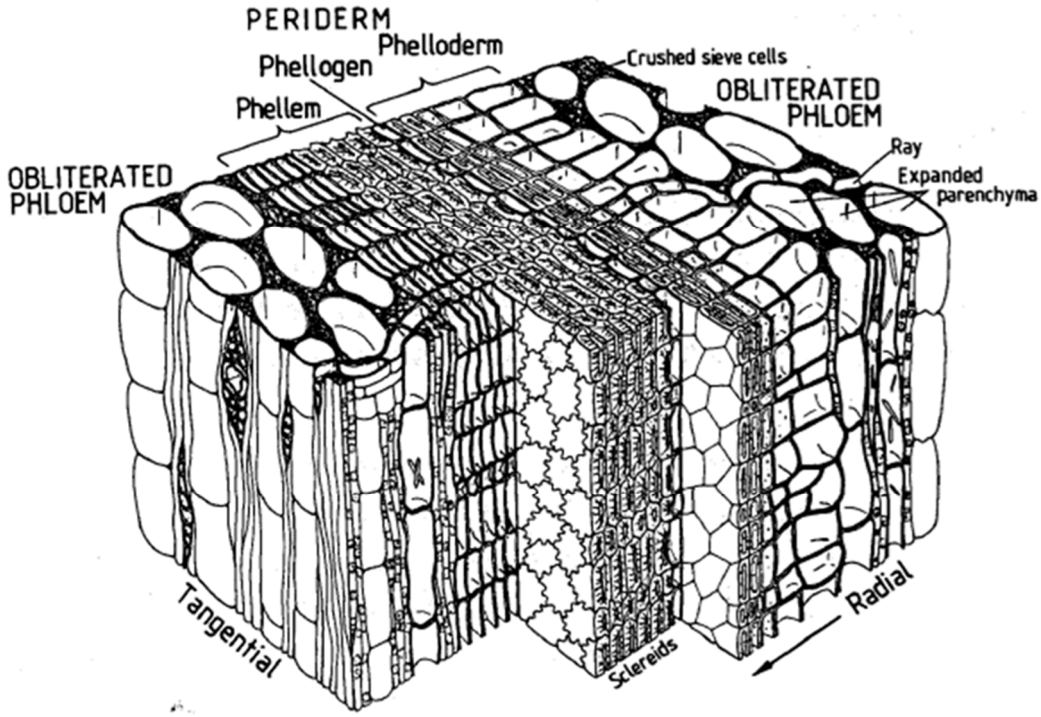

Figure 7 - Diagram of a portion of the rhytidome tissues from the stem of a typical southern pine. A single periderm, consisting of phellem, phellogen, and phelloderm is shown, on either side of which is nonconducting phloem consisting of crushed sieve cells and expanded axial parenchyma cells. The phellem consists of thin-walled cells and thick-walled cells. The phelloderm consists of unexpanded thick-walled cells and expanded thin-walled cells (Eberhardt, 2007)

The present study uses the recently adopted bark terminology IAWA List of microscopy bark features to describe the bark anatomy of Douglas-fir (Angyalossy et al., 2016):

- Rhytidome – The outer bark, which consists of the innermost periderm and tissues isolated by it, namely more peripheral periderms, phloem tissues, and – until shed – cortical tissues and epidermis;
- Periderm – Secondary protective tissue that replaces the epidermis in stems and roots, rarely in other organs. It consists of phellem (cork), phellogen (cork cambium) and phelloderm;
- Phellogen (or cork cambium) – A lateral meristem forming the periderm. It produces phellem (cork) to the outside, and phelloderm to the inside by periclinal cell divisions;
- Phellem (cork) – Protective tissue composed of nonliving cells with suberized walls and formed centrifugally by the phellogen (cork cambium) as part of the periderm;
- Phelloderm – Cells produced centripetally by the phellogen (cork cambium) as part of the periderm, often but not always resembling cortical parenchyma;
- Secondary phloem – The phloem tissue derived from the vascular cambium, composed of conducting and nonconducting phloem;
- Conducting phloem – Portion of the secondary phloem adjacent to the cambium recognized by living sieve cells with Strasburger cells;
- Nonconducting phloem – Portion of the secondary phloem recognized by sieve cells with Strasburger cells that have lost their cytoplasm and whose sieve elements are devoid of contents;
- Sieve cells – Sieve elements found in the phloem of gymnosperms with sieve areas of uniform (narrow) pore size on all walls;
- Strasburger cells – Ray and axial parenchyma cells spatially and functionally associated with the sieve cells through sieve areas;
- Axial phloem parenchyma – Parenchyma cells in the secondary phloem derived from fusiform initials of the vascular cambium;
- Phloem ray – A panel of parenchyma cells variable in height and width, formed by the ray initials in the vascular cambium and extending radially in the secondary phloem;
- Secondary phloem fibers – Elongated, tapering sclerenchyma cells derived from fusiform initials of the vascular cambium, typically undergoing intrusive growth

during development, with lignified (or less commonly unlignified – in gelatinous fibers) secondary walls;

- Sclereids – Sclelenchyma cells, variable in form and size, but typically not much elongated, with thick, often polylamellate, lignified secondary walls with many pits; in general they are derived from modification of parenchyma cells;
- Fiber-sclereids – Elongate sclereids with characteristic intermediate between those of a fiber and a sclereid; derived from axial or radial parenchyma.

2.3. Structure of Pinaceae barks

The family of Pinaceae includes 232 species in 12 genera: *Abies*, *Keteleeria*, *Cathaya*, *Pseudotsuga*, *Tsuga*, *Picea*, *Pseudolarix*, *Larix*, *Cedrus*, *Nothotsuga*, *Hesperopeuce* and *Pinus* (Farjon, 2010).

The structure of Pinaceae barks was described in several anatomical studies such as:

- Chang (1954) described the bark structure of north American conifers, mostly belonging to the Pinaceae family;
- Srivastava (1963) studied the secondary phloem of Pinaceae. He referred that in *Pinus*, *Picea* and *Larix*, the phellem consists of thin-walled cells and thick-walled cells, often arranged in alternating tangential bands of one or more cell layers; and in species of *Abies* and *Cedrus* and also in *Pseudotsuga menziesii* the phellem may consist only of thin-walled cells;
- den Outer (1967) analyzed the structure of the secondary phloem of nine families of gymnosperms including several Pinaceae species regarding the different cell types, their appearance, differentiation and degree of development: *Abies concolor*, *Tsuga canadensis*, *Cedrus libani*, *Larix decidua*, *Pseudolarix kaempferi*, *Picea* spp., *Pseudotsuga taxifolia* and *Pinus sylvestris*;
- Martin (1969) published detailed studies on the anatomy of southern pine barks (e.g. *Pinus echinata*, *Pinus taeda*, *Pinus palustris* and *Pinus rigida*);
- Howard (1971) studied the bark of 10 southern pines relating structural factors e.g. periderm shape and spacing, amount of stone cells, tangential zones of weakness and degree of obliteration and expansion with variation in physical

and mechanical properties; he identified two distinct types of cells in the phellem: suberized thin-walled cork cells and unsuberized thick-walled phellem cells;

- Barnett (1974) published a study about the structure of sieve cells and parenchyma cells of the secondary phloem in *Pinus radiata*;
- Patel (1975) studied the bark anatomy of *Pinus radiata*, *Pinus nigra*, and *Pseudotsuga menziesii*;
- Sands (1975) presented a study regarding the morphology, anatomy and chemistry of *Pinus radiata* bark;
- Bramhall et al. (1977) studied the bark thickness of *Tsuga heterophylla* at several height positions on trees from three coastal sites in British Columbia;
- Grozdits et al. (1982) and Godkin et al. (1983) studied the periderms of three north American Pinaceae: *Picea glauca*, *Tsuga canadensis* and *Abies balsamea*;
- Nunes et al. (1996, 1999) presented the anatomy of *Pinus pinaster* and *Pinus pinea* barks;
- Crivellaro and Schweingruber (2013) described the bark anatomy of six Pinaceae: *Cedrus brevifolia*, *Cedrus libani*, *Pinus brutia*, *Pinus halepensis*, *Pinus nigra* and *Pinus pinea* from the Eastern Mediterranean region, with a focus on the island of Cyprus.

As an example, Figure 8 shows the bark structure of *Pinus pinea* : transverse section of phloem and rhytidome and cell types of the secondary phloem in tangential section (Nunes et al., 1999).

As regards Douglas-fir bark structure, the first description based on light microscope observations was presented in 1954 (Chang, 1954). Grillos (1956) studied the structure of Douglas-fir bark focusing in the origin and development of the various tissue components. The structure of the secondary phloem was detailed subsequently (Grillos and Smith, 1959).

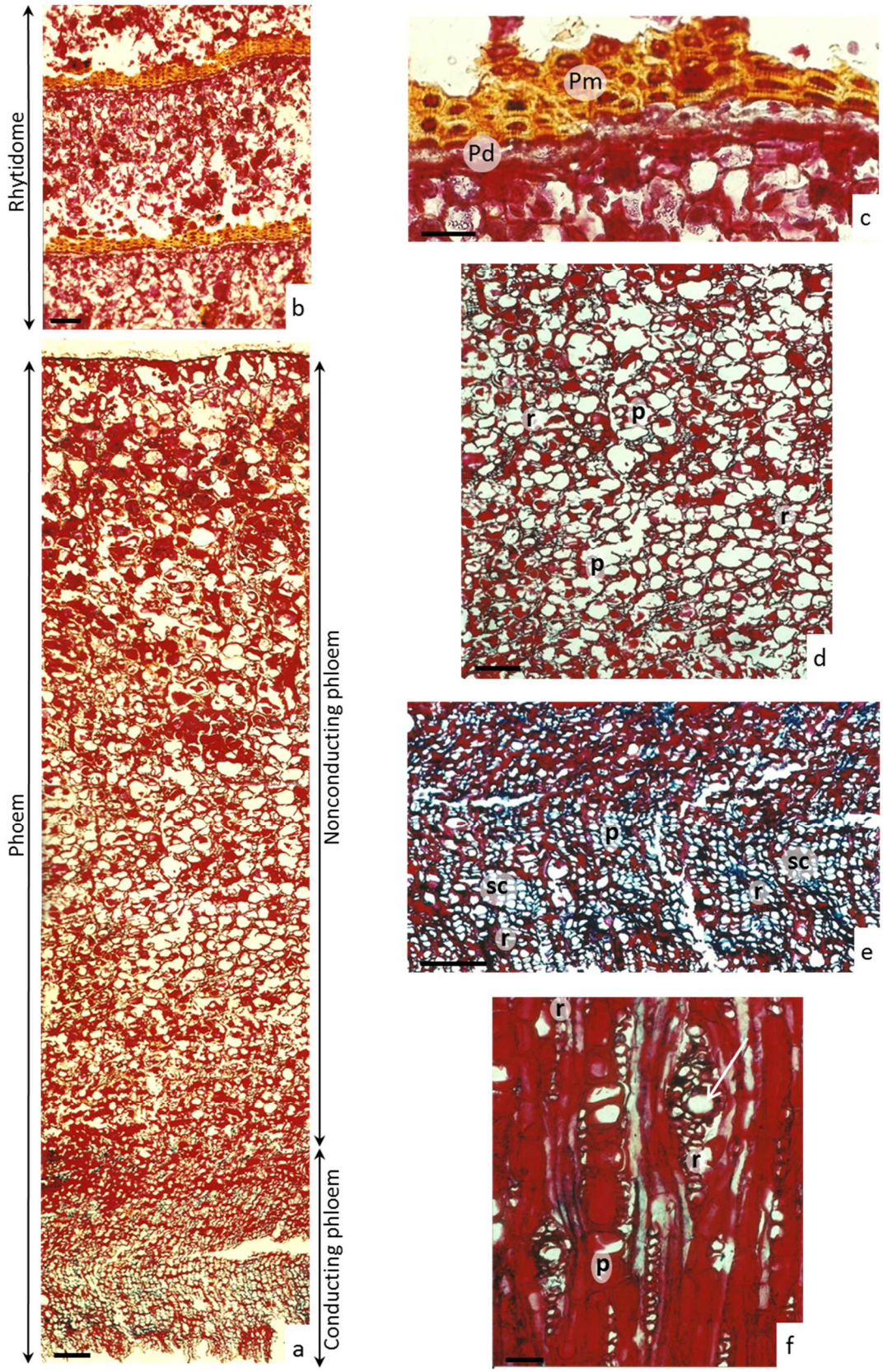

Figure 8 - Bark structure of *Pinus pinea* (Pinaceae): transverse section of phloem (a), rhytidome (b), periderm (c), nonconducting phloem (d), conducting phloem (e) and, tangential section of phloem (f) (Pm – phellem; Pd – phelloderm; p – axial parenchyma; r – parenchyma radial; sc – sclereids cells and, resin canal (white arrow)) (Bar: 200 µm (a, b, d, e); 56 µm (c, f))

Variation in structural characteristics of Douglas-fir bark regarding some anatomical and chemical characteristics of the cork and cell wall layering was also studied (Ross and Krahmer, 1971; Krahmer and Wellons, 1973; Litvay and Krahmer, 1977). Litvay (1976) presented anatomical characteristics of the Douglas-fir phellem cells by showing polarizing and ultra-violet microscopy images and also scanning electron microscopy and transmission electron microscopy images of cork cell wall structures subjected to different chemical treatments.

Dougal (1981) was the first to report a detailed illustration of Douglas-fir bark and described and illustrated ultra-structurally the anatomy of parenchyma cells present in phloem and the changes that occur in rhytidome formation as well as the pitting between sclereids and adjacent cells using stereo, light, phase contrast, fluorescence, scanning electron and transmission electron microscopy.

3. Cork characterization

3.1. Structure

One of the most interesting bark features is the presence of cork in the outer bark, because cork has a specific and rather unique set of properties that values it for various applications, of which the world known use as wine bottle stoppers (Fortes et al., 2004; Pereira, 2007). Cork is an example of one important non-wood forest products and the basis for an economic relevant production and processing chain (Pereira and Tomé, 2004).

Cork is a cellular material with a low density, very little permeability to liquids and gases, chemical and biological inertia, mechanical elasticity, high friction, good insulation and high-damping capacity (Silva et al., 2005; Pereira, 2007). The cork structure is compact with a very regular arrangement of the individual cells and without intercellular spaces. The cells are on average hexagonal prisms that are stacked base-to-base in parallel aligned radial rows. When observed in the transverse section (the plane perpendicular to the plant axis), the structure is a brick-wall type with the cells cut parallel to their prism axis and appearing with a rectangular form; the radial section is very similar. In the tangential section (the plane perpendicular to a stem radius), the cork cells appear polygonal, mostly as hexagons with a honeycomb structure (Pereira et al., 1987). Figure 9 shows the cellular structure of cork in the three sections (tangential, transverse and radial).

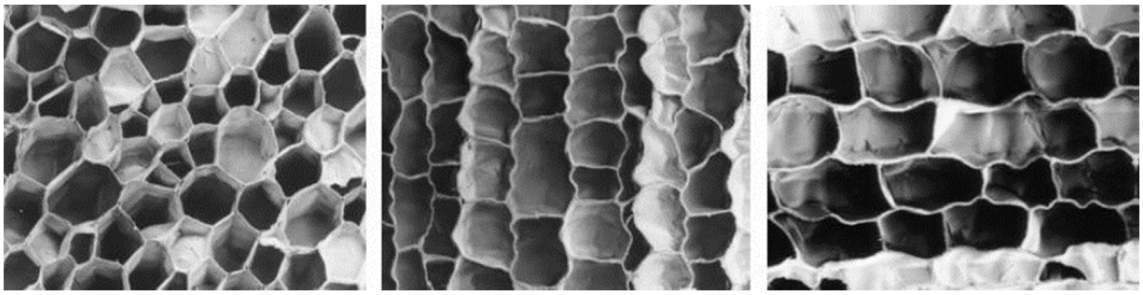

Figure 9 - Structure of cork as observed by scanning electron microscopy in the three main sections: (left) tangential section, perpendicular to the tree's radial direction; (middle) transverse section, perpendicular to the tree's axial direction; and (right) radial section, the tree's radial section (Pereira, 2015)

It is possible to identify growth increments in cork that are macroscopically distinguished by the darker color of the cell layers formed at the end of the growing season with thicker walled cells and smaller in the radial direction (latecork cells) in contrast to the thinner walls and radially longer cells of the beginning of the growing season (earlycork cells) (Pereira, 2007). The cork cells may have evenly or unevenly thickened walls, e.g., some have U-shaped wall thickenings of the inner or outer tangential wall (Evert, 2006).

The cork cells of cork oak have on average 40 µm prism height, 20 µm base edge and 1 µm wall thickness, and the cork contains about $4x10^7$-$7x10^7$ cells per cm^{-3} corresponding to a solid fraction under 20 % of the total volume (Pereira, 2007). The cell walls often show undulations, especially the lateral prism faces that in most cases have 2-3 undulations per face but that can increase to intense corrugation or to irregular patterns if subjected to stress (Pereira, 2007, 2015).

Cork is anisotropic due to the cell orientation but the shape anisotropy ratios are small, and the properties measured in the three main directions differ much less than those of wood (Pereira and Knapic, 2017).

The cork in barks of other species, although showing the general characteristics of the cork from cork oak, may differ in some structural features and in dimensions (Leite and Pereira, 2017). For instance, in *Quercus variabilis* the prism height is 22.7 µm (Ferreira et al., 2016a), smaller that the 30-40 µm registered for *Quercus suber* (Pereira et al., 1987) while it is 40-70 µm for *Kyelmeria coriacea* (Rios et al., 2014). The prism base edge in *Quercus suber* cork varied between 13-15 µm (Pereira et al., 1987), smaller than the 24 µm and 16 µm reported, respectively for *Kyelmeria coriacea* (Rios et al., 2014) and *Plathymenia reticulata* (Mota et al., 2016), and for *Quercus cerris* (Şen et al., 2011a).

3.2. Chemical composition

Cork is chemically very different from other plant tissues, namely from wood and phloem. It is out-singled by the presence of suberin as a major cell wall structural component. Cork of cork oak has the following average chemical composition: 16 %

extractives, 43 % suberin, 22 % lignin and 19 % cellulose and hemicelluloses (Pereira, 2013) (Table 1).

Table 1 - Summative chemical composition (% o.d. cork mass), monosaccharide composition (% of total neutral sugars), and proportion of cell wall structural components of cork (% of the structural components mass) (Pereira, 2015)

	% on OD Cork Mean (std)	% of Structural Components
Extractives, Total	16.2 (3.9)	
Dicholoromethane	5.8 (0.8)	
Ethanol	5.9 (3.0)	
Water	4.5 (1.6)	
Suberin, Total	44.8 (6.2)	52.8 (7.3)
Long Chain Lipids	41.0 (5.2)	48.3 (6.1)
Glycerol	3.8 (0.6)	4.5 (0.7)
Lignin, Total	22.0 (3.3)	25.9 (3.9)
Klason Lignin	21.1 (3.3)	24.9 (3.9)
Acid Soluble Lignin	0.9 (0.2)	1.0 (0.2)
Monosaccharide Composition (% of Total Neutral Sugars)		
Glucose	46.1 (3.6)	
Xylose	25.1 (3.7)	
Arabinose	18.0 (3.0)	
Mannose	3.0 (2.8)	
Galactose	7.3 (1.2)	
Rhamnose	0.5 (0.5)	

The chemical composition of corks from other species show some differences when compared to *Quercus suber* cork, namely regarding the suberin content (Leite and Pereira, 2017). For instance, the cork of *Quercus variabilis* presented 38.1 % suberin (Ferreira et al., 2016a), *Kyelmeria coriacea* 16.1-30.3 % (Rios et al., 2014), *Plathymenia reticulata* 24.7 % (Mota et al., 2016), *Quercus cerris* 28.5 % (Şen et al., 2010) and *Betula pendula* 36.2 % (Ferreira et al., 2017), smaller than the 42.8 % registered for *Quercus suber* cork (Pereira, 2013).

Suberin is a large biopolymer of lipid nature formed by the esterification of glycerol and long-chain fatty acids, α,ω-diacids and ω-hydroxyacids, either saturated or with an unsaturation, epoxy, or vicinal diol substitution at mid-chain (Graça and Pereira, 1997). The content of suberin is the most important chemical attribute of cork since it is directly related to most of its typical properties; lignin is the second most important structural component and also determines the behavior of the material (Pereira, 2015). Contrarily

to what happens in wood, cellulose has a lesser role in the construction of the cork cell wall and for the material's properties (Pereira, 2013).

Lignin is a macromolecule, a cross-linked aromatic polymer with strong covalent bonds disposed as a 3D-network that confers strength to the cell wall (Pereira, 2007). Lignin is usually defined as a polymer of phenylpropane units derived from three percursors (p-coumaryl alcohol, conyferyl alcohol and synapyl alcohol) with three different aromatic units — p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) (Figure 10). The lignins are classified according to their H/G/S ratios (Lourenço and Pereira, 2018). Lignin structural composition of barks, namely of corks, is largely unknown except for a few cases that showed that cork lignin is composed mainly of guaiacyl units with a low proportion of syringyl units (Marques et al., 1994, 1996, 1999, 2006, 2016; Marques and Pereira, 2013).

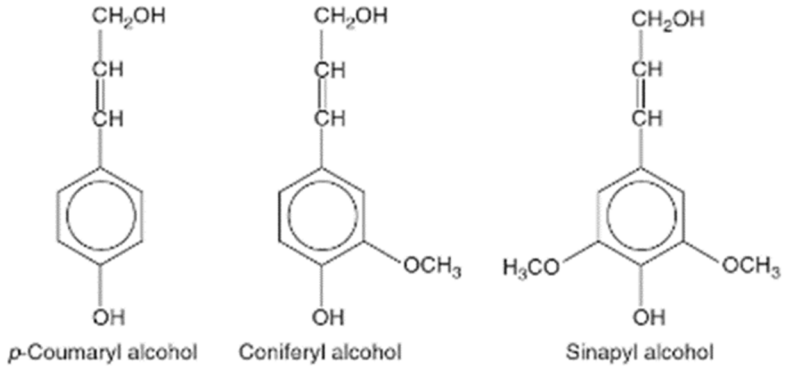

Figure 10 - Monomeric structures of the three lignin percursors p-coumaryl alcohol, coniferyl alcohol and synapyl alcohol that are at the origin of the H, G and S units, respectively of lignin (Pereira, 2007)

The structural polysaccharides of cell walls are cellulose and hemicelluloses. While in wood they represent up to 80 % of the structural components of the cell wall, in cork they have a much lower importance and correspond to about 20 % of cork (Silva et al., 2005; Pereira, 2007). Xylans are the most important hemicelluloses in cork (Pereira, 1988).

Cork also contains non-structural components that are soluble in different solvents. Lipophilic extractives including fatty acids and alcohols, sterols, and terpenes, as well as polar compounds of phenolic nature are present in substantial amounts. The proportion

and the composition of cork extractives differ substantially between species (Ferreira et al., 2015, 2016a, 2016b, 2017; Mota et al., 2016; Sen et al., 2016). The inorganic materials content, determined as ash, is usually below 3 % in cork (Pereira, 1988; Şen et al., 2010; Ponte-e-Sousa and Neto-Vaz, 2011; Ferreira et al., 2016a, 2016b).

Much effort has been undertaken to study the variability of *Quercus suber* cork in relation to chemical composition because this characteristic is responsible for many of its properties (Pereira, 1988, 2013; Bento et al., 2001; Sen et al., 2016). For corks of other species, there is no systematic study of natural chemical variation.

Together, the cell structure and chemical composition determine cork properties, e.g., the solid volume ratio and the material's density that influence elasticity and mechanical strength, as well as cork performance in insulation (Pereira, 2015). Of all mechanical properties, compression behavior is the one that has attracted most attention, due to the importance of compression in the use of cork as stoppers for wine bottles (Anjos et al., 2008, 2014; Oliveira et al., 2014).

3.3. Barks with cork

The main provider of cork is the cork oak (*Quercus suber*) and it has been extensively studied, as reviewed by Pereira (2007). This species produces a thick bark with a continuous layer of cork tissue on the outside, with a thickness and properties that make it a valuable raw material for industry. When the cork is removed, by separation at the phellogen, the tree has the capacity to form a new periderm and add new layers of cork every year, and this may be repeated throughout the tree's lifetime allowing the sustainable production of cork (Pereira and Tomé, 2004).

Other species are referred as having corky barks, as compiled recently by Leite and Pereira (2017) who classified these species according to their bark structure: presence or absence of rhytidome (Table 2).

Table 2 - List of gymnosperm and angiosperm species that have been studied in relation to their cork-rich barks, classified according to their bark structure (presence/absence of rhytidome) (Leite and Pereira, 2017)

	Bark with rhytidome	**Bark without rhytidome**
Gymnosperm	*Pseudotsuga menziesii* *Abies lasiocarpa* var. *arizonica* *Abies concolor*	
Angiosperm	*Quercus cerris* *Betula pendula*	*Quercus suber* *Quercus variabilis* *Kielmeyera coriacea* *Plathymenia reticulata*

Some studies have characterized the structure and chemical composition of cork from different species: *Quercus variabilis* (Miranda et al., 2013b; Ferreira et al., 2016a), *Quercus cerris* (Şen et al., 2010, 2011a), *Kielmeyera coriacea* (Rios et al., 2014), *Betula pendula* (Pinto et al., 2009; Miranda et al., 2013a; Ferreira et al., 2017) and *Plathymenia reticulata* (Mota et al., 2016).

Other species have also been referred as having a substantial proportion of cork in their barks but their cork has not been characterized: *Quercus occidentalis* (Roth, 1981), *Abies lasiocarpa* var. *arizonica* (Natividade, 1950), *Abies concolor* (Natividade, 1950), *Phellodendron amurense* (Natividade, 1950), *Ulmus campestris* var. *suberosa* (Natividade, 1950), *Erythrina* sp. (Natividade, 1950), *Melaleuca leucadendron* (Natividade, 1950), *Pithecolobium incuriale* (Natividade, 1950; Abramovay, 1999), *Enterolobium ellipticum* (Natividade, 1950; Roth, 1981), *Aspidosperma tomentosum* (Natividade, 1950), *Zeyheria montana* (Natividade, 1950), *Connarus suberosus* (Natividade, 1950), *Agonandra brasiliensis* (Rizzini and Mors, 1995), *Pisonia tomentosa* (Rizzini and Mors, 1995), *Aspidosperma dasycarpum* (Rizzini and Mors, 1995), *Erythrina mulungu* (Rizzini and Mors, 1995), *Symplocos lanceolate* (Rizzini and Mors, 1995), *Erythrina crista-galli* (Abramovay, 1999), *Stryphnodendron adstringens* (Roth, 1981; Abramovay, 1999) and *Anona coriacea* (Roth, 1981; Abramovay, 1999).

Original research

1. Research outline

The research carried out in this work aimed at studying the development of Douglas-fir bark in trees grown in Portugal, and at characterizing its cork component in relation to features that are relevant for its valorization. The objective is to assess the potential use of Douglas-fir as a cork provider by incorporating the bark component in an integrated forest use of the species. While aiming at a general valorization of the whole tree, under a full resource use approach, in the case of Douglas-fir the bark has a substantial proportion of cork in the outer bark of older trees that allows considering its fractionation and use in cork-based products.

To achieve this objective it was necessary to known how bark develops in the Douglas-fir trees, including the age related cork development, as well as the structural and chemical features of the bark and most specially the cork features that are relevant for application.

A sampling of Douglas-fir trees grown in Portugal was made in two sites at the time of harvest for the saw milling industry. A collection of stem discs was made along different height levels of the stem e.g. corresponding to different ages from 20 trees. These samples were the study material used in the following four tasks:

1. The stem development was studied regarding the age related development of heartwood, sapwood and bark including variation with cambial and tree age, as well as on an analysis of ring width and taper of the trees planted in northern and central regions of our country. The results will also give support to potential afforestation plans with this species and contribute to an increased valuation of Douglas-fir stem utilization. The results are presented in Publication I.

2. The cork of the Douglas-fir bark was studied in detail regarding its structural and anatomical features, including cell biometry, in view of evaluating its potential for cork products since the structural features of cork are determining to its use as a cellular material. The results are presented in Publication II.

3. Since Douglas-fir bark contains a significant cork content in the rhytidome, the study of the bark structural development with age and the rhytidome

development along the stem was made, focusing on the cork component in relation to the cambial age. The results are important to assess the quantitative potential of Douglas-fir as a cork provider. The results are presented in Publication III.

4. The chemical composition of the bark of Douglas-fir was studied in relation to its age, including the composition of the cork component. The main objectives are to contribute for the potential utilization of Douglas-fir bark residues through the valorization of the cork component. The summative composition (extractives, lignin, suberin, polysaccharides and ash) was analysed as well as the specific composition of suberin and polysaccharides. The results are presented in Publication IV.

2. Material and methods

2.1. Sampling

The selection of the sampling sites and collection of tree samples were conditioned by some administrative and logistic factors external to the study. Since most of Douglas-fir plantations in Portugal are state properties, the access is only given after trees are sold to sawmills.

The samples were collected from two state-owned stands in northern and central Portugal at the time of tree harvest for the saw milling industry. One stand was located in the Forest Perimeter of Serra da Cabreira, Cabeceiras de Basto (40°21'28.5''N, 07°27'07.2''W), and the other stand in the Forest Perimeter of Sarzedo, in the region of Serra da Estrela, Covilhã (41°35'18.0''N, 8°01'00.6''W). The stands are named Cabreira and Estrela, respectively. Cabreira is a mixed and irregular stand and Estrela is a pure and regular stand (Figure 11). In both sites, silvicultural operations have not been made in recent years due to landscape preservation. Table 3 gives a summary of the main edapho-climatic classification of stands.

Figure 11 - Identification and localization of two sampling sites Cabreira (C) and Estrela (E) in Portugal

Table 3 - Main edapho-climatic classification of the sampled stands of Douglas-fir

	Cabreira	Estrela
Altitude (m)	850	930
Annual precipitation (mm)	1600-2000	1400-1600
Annual mean temperature (°C)	7.5-10.0	
Soils	Rankers	Cambisols

From the trees signaled to harvest, ten trees were randomly selected in each stand and characterized by measuring total height, crown base height (c.b.h.) and diameter at 1.3 m above ground (d.b.h.), as the mean of two crossed diameters. The age of the trees was estimated by ring counting at the stump cut at ground level (or stem base). The biometric information of sampled trees is presented in Table 4.

Both stands were harvested in 2015, Cabreira in January and Estrela in September, to produce 2.5 m long logs for the sawmilling industry. Stem discs with approximately 10 cm thickness were taken at the bottom end of each log totaling 212 stem discs (Figure 12). The stem discs were air dried in-door under well ventilated conditions. Care was taken to maintain all the bark of the stem discs.

For the detailed observation of the cork cellular features by scanning electron microscopy (SEM) observations, additional bark samples were collected from trees with approximately 100 years of age, grown in the central mountain of Serra da Estrela, in Portugal. The barks were stored in indoor conditions with low light and good ventilation.

Table 4 - Tree age, overbark diameter at 1.3 m (d.b.h.), total height and crown base height (c.b.h.) of the 20 Douglas-fir trees sampled in the two sites (Cabreira an Estrela) (Mean of 10 trees ± standard deviation)

	Cabreira				Estrela			
Tree	Age (years)	d. b. h. (cm)	Height (m)	c.b.h. (m)	Age (years)	d. b. h. (cm)	Height (m)	c.b.h. (m)
1	46	61.5	30.2	5.8	49	55.7	32.9	2.5
2	46	73.6	35.7	2.7	48	60.5	35.6	2.5
3	45	57.3	27.9	3.4	48	54.5	34.0	2.5
4	43	58.6	23.7	5.5	39	73.9	33.1	1.0
5	46	57.3	29.5	6.0	49	65.3	30.5	2.0
6	44	57.3	25.7	3.3	46	65.0	46.1	2.0
7	50	63.7	29.4	5.0	46	56.7	35.4	5.0
8	45	54.1	29.5	4.6	64	53.8	35.5	4.4
9	44	57.3	27.7	3.1	53	64.3	35.6	2.5
10	48	66.9	31.9	6.3	59	58.9	30.4	2.5
Mean	45 ± 2	60.8 ± 5.8	29.1 ± 3.3	4.6 ± 1.3	50 ± 7	60.9 ± 6.3	34.9 ± 4.4	2.7 ± 1.3

Figure 12 - Height sampling scheme and stem discs collected from one tree

2.2. Stem characterization

The surface of the stem discs was smoothed by sanding. In all cases, the annual rings were distinct, and the heartwood was clearly recognizable with an orange/reddish color that contrasted to the pale sapwood.

The age of each disc as the total number of annual rings and the number of rings included in the heartwood and sapwood were counted in two opposite radii (Appendix I). The measurement of heartwood, sapwood and bark radial widths were made in eight radial directions approximately evenly spaced randomly marked on the cross-section of discs (Figure 13) (Appendix II). The average value was used for calculation of their areas considering a circular stem disc. The following calculations were made for heartwood and sapwood for each stem disc: radial width (cm), area (cm^2) and proportion of the wood disc (heartwood and sapwood area in percent of total wood disc area). For bark,

the following calculations were made: thickness (mm), area (cm^2) and proportion of stem disc (bark area in percent of total stem disc area).

The width of annual rings was measured from pith to bark along two opposite radii using Analysis software (version 3.2, AnalySIS Soft Imaging System GmbH, Munster, Germany) (Figure 13). The arithmetic mean of the measurements was used in the calculations of tree growth. Latewood width in each ring was also measured and its proportion was calculated (Figure 13).

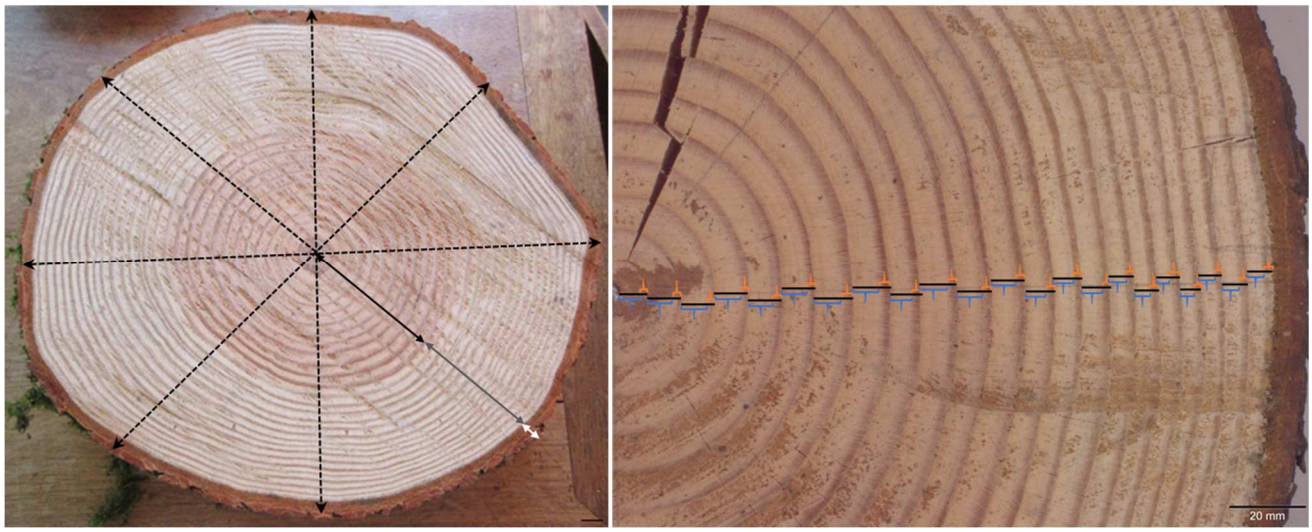

Figure 13 - Sample with eight radial directions considered to determine the radial width of sapwood (grey arrow) and heartwood (black arrow) and thickness of bark (white arrow). Example of measure of one stem disc width of annual rings (black line) and with distinction of earlywood (blue line) and latewood (orange line)

2.3. Bark structural and anatomical characterization

The bark structural development was studied on the cross-sectional discs from each tree along the stem at six height levels at intervals of approximately 5 m of stem height that corresponded to a difference of ca. 6 years of tree age between height levels.

Images of the cross-sectorial discs were acquired and processed using the Analysis software. The determination of the radial thickness of phloem, periderm or rhytidome in the bark and proportion of phloem and cork in rhytidome was made on two opposite radii. Using the same images, in three randomly directions, the number of periderms in

the rhytidome was determined and the thickness of the phloem and cork layers in the rhytidome measured with the Leica Qwin Plus software.

For anatomical observations, bark specimens were taken at each height level and were impregnated with DP 1500 polyethylene glycol and transverse and longitudinal microscopic sections of approximately 17 µm thickness were prepared with a Leica SM 2400 microtome using Tesafilm 106/4106 adhesive for sample retrieval. The sections were stained with a double staining of chrysodine and astra blue. The sections were mounted on glycerine Kaiser and after 24 h drying, the lamellas were submerged in xylol during 30 min to remove the Tesafilm, dehydrated on 96 % and 100 % ethanol and mounted on Eukitt. Individual specimens of bark samples were also macerated in a 1:1 acetic acid and hydrogen peroxide solution at 60 ^{o}C for 48 h for cell dissocion and stained with astra blue.

The parameters measured and the numbers of measurements for each specimen were as follows: length, width and cell wall thickness of 40 fibre-sclereids, measured in the macerated material; length and tangential diameter of 30 sieve cells measured in the macerated material and in the transverse section, respectively; and rays measured in a field of the tangential section (objective of 4x). All measurements were made using a microscope and a semiautomatic image analyzer system (Leica Application Suite). The proportion of cell types was measured in the transverse sections using a 48-point grid on five areas from phloem to rhytidome. The light microscopic observations were made using a Leica DM LA microscope and photomicrographs were taken with a Nikon Microphot-FXA.

The terminology follows mainly IAWA List of microscopic bark features (2016). Small cubes of bark with approximately 5 mm of edge were cut with a sharp razor blade and the surfaces were observed with a scanning electron microscope Hitachi TM 3030 Plus to obtain images recorded in digital format.

2.4. Structural and anatomical features of cork

For the study of structural and anatomical features of cork of Douglas-fir bark the samples were collected from the bottom part of the trees, from the base up to 1.3 m of

stem height. The transverse sections of the bark rhytidome were observed by image analysis system and the area proportion of cork was calculated using that image analysis system software.

Small cubes with approximately 3 mm of edge were cut with a sharp razor blade in the cork region within the rhytidome and were observed under electron scanning microscope (SEM). For those observations the cubes were mounted on stubs (ProSciTech, Australia) and sputter coated (Polaron E 5100 E, USA) with gold palladium for 3 min at 20 mA. The transverse, tangential and radial sections surfaces were observed in an SEM Hitachi S-2400 at magnifications ranging from 50 to 1000×, and the images were recorded in digital format.

In the SEM images corresponding to the different sections (transversal, tangential and radial) the cell measurements were made using image analysis software (Leica Qwin Plus). The measurements were averaged for the tangential section (the honeycomb type cellular arrangement) and the non-tangential sections (the brick-wall type of structure).

For characterizing the cork tissue, the number of edges of each cell i.e. the number of neighboring cells, was counted on the tangential and non-tangential sections. The distribution function of the number of edges of each cell was calculated based on the results of a total of 400 cells for each section as $f_i = N_i / \sum N_i$ where N_i represents the number of cells with i edges and $\sum N_i$ the total number of cells. The topological disorder of the bi-dimensional networks was evaluated by the dispersion of the function in relation to the mean $i_m$was calculated as $\mu_2 = \sum (i - i_m)^2 f_i$.

The average cell area was measured on the tangential sections, corresponding to the average prism base area, and the cell prism height was measured on the non-tangential sections (Figure 14). The cell wall thickness was measured in tangential and non-tangential sections as the radial dimension (Figure 14). A total of 400 cells were measured for each section. This type of measurements was only possible in the tissue regions where the cells were not corrugated or crushed, therefore representing the potential non-compressed cellular tissue.

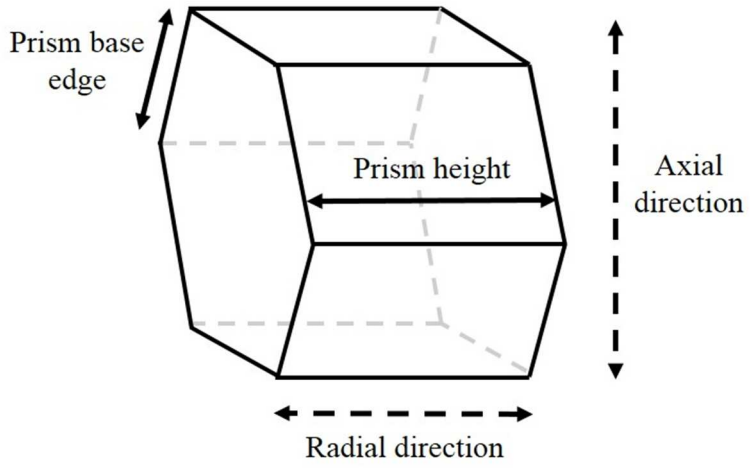

Figure 14 - Schematic drawing of a cork cell as a hexagonal prism showing the axial and radial directions and indicating where prism base edge and prism height were measured

2.5. Bark chemical characterization

For determining the chemical composition, bark samples of six trees, three from each site were used. The bark was manually removed from the discs at three height levels (base, middle and top) along the stem tree, corresponding to a variation of approximately 14 years of tree age between height levels in intervals of 10 in 10 m of height.

In the bark samples from the base level, the phloem and the rhytidome were separated, and the cork and phloem portions were separated from the rhytidome. First the phloem and the rhytidome were separated manually with a chisel and hammer and then the cork and the phloem portions of the rhytidome were separated with a x-act from one tree of each site.

The whole bark was analysed at the three height levels.

The bark, phloem and cork samples were ground in a cutting mill (Retsch SM 2000 - using an output sieve with 10 mm x 10 mm openings, for bark and, Retsch CEP 405, for cork) and sieved with a vibratory sieving apparatus (Retsh AS 200 basic) with standard sieves with the mesh sizes 80 (0.180 mm), 60 (0.250 mm), 40 (0.425 mm) and 20 (0.850 mm). The 40–60 mesh fraction was used for chemical analysis. All determinations were made with duplicate samples.

The chemical summative analyses included determination of ash, extractives soluble in dichloromethane, ethanol and water, suberin, klason and acid soluble lignin, and the monomeric composition of polysaccharides.

Ash was determined by measuring the residue remaining after incinerating the sample in a muffle furnace at 525ºC.

The extractives were determined with procedures adapted from TAPPI 204 cm-97, in a Soxhlet system successively with dichloromethane (6 h), ethanol (16 h) and water (16 h). The extractives solubilized by each solvent were determined by mass difference of the solid residue and reported as percent of the original sample.

The suberin content was determined in the extractive-free material of the whole bark and cork samples (suberin was not determined in the phloem samples) by use of methanolysis for depolymerization. A 1.5 g sample of extractive-free material was refluxed with a 3 % (m/v) solution of $NaOCH_3$ in CH_3OH (100 ml) during 3 h. The sample was filtrated and washed with methanol, and the filtrated residue was refluxed again with 100 ml CH_3OH for 15 min and filtrated. The combined filtrates were acidified to pH 6 with 2 M H_2SO_4 and evaporated to dryness. The residues were suspended in water (50 ml) and the products recovered with dichloromethane in three successive extractions (of 50 ml each). The combined extracts were dried over anhydrous Na_2SO_4, and the solvent evaporated to dryness. The suberin extracts were quantified and the results expressed in percent of the initial dry mass. It should be noticed that the suberin extracts include the long chain acids, alcohols and the other monomers that are soluble in dichloromethane while glycerol, also a monomer of suberin, is lost in the water fraction.

Klason and acid-soluble lignin, and carbohydrates contents were determined on the extracted and desuberinized materials. Sulphuric acid (72 %, 3.0 ml) was added to 0.35 g of the sample and the mixture placed in a water bath at 30 ° C for 1 h. The solution was diluted with water until the sulfuric acid concentration was 3 % and then autoclaved at 121 ° C for 30 min. After cooling, the insoluble fraction was separated by filtration and the Klason lignin was weighed after drying at 105 ° C and the acid-soluble lignin determined by measuring the UV absorption at 206 nm using an extinction coefficient of 110 $l\ g^{-1}\ cm^{-1}$. The remaining acid solution was kept for sugar analysis. The

composition of polysaccharides was evaluated by determining the content in neutral monossacharides (rhamnose, arabinose, xylose, galactose, mannose and glucose) and uronic acids (galacturonic and glucuronic acids) in the hydrolysate from the lignin analysis using High Pressure Ion-exchange Chromatography with a pulsed amperometric detector (HPIC-PAD). The compounds were separated in a Dionex ICS-3000 system, with an Aminotrap plus Carbopac PA10 column (250 x 4 mm). The content of acetic acid was also determined in the hydrolysate using a High-Pressure Ionexclusion Chromatography with a UV/Visible detector (HIPCE-UV). The compounds were separated in a Thermo Finnigan Surveyor installed with a Biorad Aminex 87H column (300 x 7.8 mm).

The suberin composition was determined taken aliquots of the dichloromethane extracts (5 ml) from the suberin depolymerization reaction and evaporated it under N_2 flow and dried at room temperature under vacuum overnight. The samples were derivatized prior to analysis: they were dissolved in 120 μL of pyridine and the compounds with hydroxyl and carboxyl groups were trimethylsilylated into trimethylsilyl (TMS) ethers and esters, respectively, by adding 80 μL of bis(trimethylsily)-trifluoroacetamide (BSTFA). The reaction mixture was heated at 60 ° C for 30 min in an oven and immediately analyzed by injection in a GC-MS Agilent 5973 MSD with the following GC conditions: Zebron 7HG-G015-02 column (30 m, 0.25 mm; ID, 0.1 μm film thickness), flow 1 ml min^{-1}, injector 280 ° C, oven temperature program, 100 ° C (1 min), rate of 8 ° C min^{-1} up to 250 ° C, rate of 5 ° C min^{-1} up to 300 ° C (5 min), rate of 5 ° C min^{-1} up to 350 ° C (5 min), rate of 10 ° C min^{-1} up to 380 ° C (5 min). The MS source was kept at 220 ° C and the electron impact mass spectra (EIMS) taken at 70 eV of energy. The compounds were identified and quantified as TMS derivatives by comparing their mass spectra with a GC-MS spectral library (Wiley, NIST), and by comparing their fragmentation profiles with published data, reference compounds, ion fragmentation patterns, and/or retention times. Each aliquot was injected in triplicate and results presented by mean (only standard deviation inferior to 5 % was considered).

Publication I.

Characterization of Douglas-fir grown in Portugal: heartwood, sapwood, bark, ring width and taper

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ORIGINAL PAPER

Characterization of Douglas-fir grown in Portugal: heartwood, sapwood, bark, ring width and taper

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Abstract Douglas-fir (*Pseudotsuga menziesii*) is one of the best timber conifers providing long sawnwood components. Original from North America, it has been planted in Europe on approximately 550 thousand ha. Twenty Douglas-fir trees growing in two sites in Portugal were studied regarding ring analysis, heartwood, sapwood and bark development, and taper. The radial growth rate was 7.1 and 6.6 mm year^{-1} at stem base for 45- and 50-year-old trees, respectively, in the two sites. Initial growth rate was slower, increasing until about 20 years and decreasing afterwards. Heartwood proportion represented on average 49% of the cross section in the lower part of the stem and decreased upwards. Heartwood formation was estimated to start at a cambial age of 8–9 years and increasing by 0.7–0.9 rings year^{-1}. Sapwood width was on average 75 mm at stem base, decreasing upwards. Bark was 26–27 mm thick at stem base, where it represented 15% of the cross-sectional area and decreased to 3–5 mm at the top. Stemwood and heartwood tapers were on average 15 mm m^{-1} in the lower stem part and 21 and 18 mm m^{-1}, respectively, in the upper part. Douglas-fir showed a good potential for the mountain areas of Portugal, and under the silvicultural conditions of both stands the trees presented ring homogeneity, small conicity and low taper suitable for long wood components.

Keywords Douglas-fir · Heartwood · Sapwood · Bark · Ring width · Taper

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Introduction

Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), a species native to North America where it has a wide distribution, was introduced outside its natural range in various regions, namely in Europe nearly 200 years ago, where it is now the most widely distributed North American conifer (Lavender and Hermann 2014). Douglas-fir occupies approximately 19 million ha in the USA and Canada (Weiskittel et al. 2012) and over 550 thousand ha in Europe. Most timber comes from plantation forests in North America and Europe that provide sawn timber of great length and quality due to the large stem size and excellent wood properties (Ross and Krahmer 1971). Douglas-fir is now the economically most important exotic timber tree species in European forests (Schmid et al. 2014). In Portugal, Douglas-fir covers about 4200 ha (Martins 1999), but the potential area where this species could be planted is estimated at 250,000 ha (Fontes 2002).

The stem quality is a determining factor for the economic and technological performance of trees when they are intended for high-value timber. Stem quality parameters such as the radial and axial development of ring width, tree taper values, as well as heartwood proportion and its development in height, are usually considered. Heartwood is generally preferred for most timber applications because of its higher natural durability, darker colour and aesthetic value (Pereira et al. 2003; Sousa et al. 2013). Heartwood formation and development vary between species and within a species with growth rate, stand and tree biometric features, site conditions and genetics, while sapwood radial

width is associated with heartwood formation and accumulation (Bamber and Fukazawa 1985; Hillis 1987; Taylor et al. 2002). Knowledge on heartwood and sapwood development over time at different heights of the trees is therefore a reference information for establishing the technological quality of a timber species, as studied for several species, e.g. *Pinus pinaster* (Pinto et al. 2004; Knapic and Pereira 2005), *Tectona grandis* (Miranda et al. 2011), *Quercus faginea* (Sousa et al. 2013), *Acacia melanoxylon* (Knapic et al. 2006).

Despite the economic importance of Douglas-fir, little information exists on its heartwood development as a technological stem quality parameter, and more information is available on sapwood development (Gartner 2002; Hein et al. 2008; Domec et al. 2012). This is addressed in the present study that analyses the within-tree development of heartwood and sapwood in relation to tree and cambial ages.

Bark proportion was also analysed since large quantities of Douglas-fir bark will be available as a residue from the primary processing of logs at sawmills or other processing mills. Douglas-fir bark is an interesting biomass component, namely because it contains a substantial proportion of cork. This potential was recognized in early studies (Kurth 1950; Hall 1971; Krahmer and Wellons 1973; Patel 1975), and recent work further investigated Douglas-fir bark as a chemical source for biorefineries (Ferreira et al. 2015, 2016).

This paper reports on the stem quality of mature Douglas-fir trees at the time of harvest in two plantations in Portugal. It is our objective to analyse their technological quality regarding ring analysis, heartwood, sapwood and bark development from a sawmilling targeted perspective. The aim is also to contribute for an integrated valuation of this timber species that is considered to have a good potential for the mountain areas of the country.

Materials and methods

The study was based on 20 Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) trees sampled from two state-owned stands in northern and central Portugal. The stands resulted from the afforestation carried out by the state in the mid of the last century. Although the seed origin is not known, isozyme analysis suggested that the plantations are originated from seed from Oregon, west of the Cascade Range, therefore of the coastal variety *Pseudotsuga menziesii* var. *menziesii* (Fontes 2002). The afforestation was made using the forest services practices at the time for these conditions, namely targeting dense forests with densities around 1000–1200 trees ha^{-1}. There are no records of silvicultural management.

One stand (here named Cabreira), located in the Forest Perimeter of Serra da Cabreira, Cabeceiras de Basto (40°21′28.5″N, 07°27′07.2″W, 850 m of altitude, 7.5–10.0 °C annual mean temperature, 1600–2000 mm annual precipitation and Rankers soils), is a mixed stand with Douglas-fir as the dominant species but including also the softwoods *Pinus pinaster, Pinus sylvestris, Cupressus lusitana* and *Chamaecyparis* spp., and the hardwood *Betula pendula* with an average 1100 trees ha^{-1}. The other stand (named Estrela), located in the Forest Perimeter of Sarzedo, in the region of Serra da Estrela, Covilhã (41°35′18.0″N, 8°01′00.6″W, 930 m of altitude, 7.5–10.0 °C annual mean temperature, 1400–1600 mm annual precipitation and Cambisols soils), is a pure and regular stand with an average 1200 trees ha^{-1}.

Both stands were harvested to produce logs for the sawmilling industry. At the time of felling, ten trees were randomly selected in each stand and characterized by measuring total height, crown base height and diameter at 1.3 m above ground (d.b.h., as the mean of two crossed diameters). Tree age ranged from 43 to 50 years and 39 to 64 years, respectively, at Cabreira and Estrela, as given by ring counting at the stump cut at ground level (here called stem base), tree height averaged 29 m and 35 m, overbark diameter at breast height (d.b.h) ranged between 54 and 74 cm and the crown base of height to the first branch (c.b.h.) ranged between 1 and 6 m (Table 1). Measurement of the living crown height was not taken.

The trees were bucked into 2.5-m-long logs in accordance with the sawmill's demands. Stem discs (10 cm thick) were taken at the bottom end of each log, totalling 212 stem discs. The stem discs were air-dried in-doors and under well-ventilated conditions. The surface of the stem discs was smoothed by sanding. In all the cases, the annual rings were distinct and the heartwood was clearly recognizable by an orange/reddish colour that contrasted to the pale sapwood.

Eight radial directions approximately evenly spaced were randomly marked on the cross section for measurement of heartwood, sapwood and bark radial widths. The average value of these measurements was used for

Table 1 Tree age (as the number of rings at stem base), overbark diameter at 1.3 m (d.b.h.), total height and crown base height (c.b.h.) of the Douglas-fir trees sampled in the two sites of Cabreira and Estrela

	Cabreira	Estrela
Age (years)	45 ± 2 (43–50)	50 ± 7 (39–64)
d.b.h. (cm)	60.8 ± 5.8 (54.1–73.6)	60.9 ± 6.3 (53.8–73.9)
Height (m)	29.1 ± 3.3 (23.7–35.7)	34.9 ± 4.4 (30.4–46.1)
c.b.h. (m)	4.6 ± 1.3 (2.7–6.3)	2.7 ± 1.3 (1.0–5.0)

Mean of 10 trees ± SD and in parentheses interval of variation

calculation of their corresponding areas considering a circular stem disc. The total number of annual rings and the number of rings included in the heartwood and sapwood were also determined.

The following variables were calculated for each stem disc:

(a) for bark: thickness (mm), area (cm^2) and proportion of the stem disc (bark area in per cent of total stem disc area);
(b) for heartwood: radius and diameter (cm), area (cm^2), proportion of the wood disc (heartwood area in per cent of total wood disc area) and number of rings;
(c) for sapwood: radial width (cm), area (cm^2), proportion of the wood disc (sapwood area in per cent of total wood disc area) and number of rings.

Volumes of tree (outside bark), stemwood (inside bark) and heartwood up to the commercial height were calculated using sections corresponding to the different heights of sampling as conical sections, and a cone (top above the last commercial log), using the following equations (Gominho and Pereira 2000):

$$V = \frac{h}{3}\left(\mathrm{Sa} + \mathrm{Sb} + \sqrt{\mathrm{Sa} \times \mathrm{Sb}}\right)$$

where Sa is the area at the lower height level; Sb is the area at the higher level; h is the length of the section. Sapwood volume was calculated as the difference of stemwood and heartwood volumes. Bark volume was calculated as the difference of tree and stemwood volumes.

The width of annual rings was measured from pith to bark along two opposite radii using Analysis software (version 3.2, AnalySIS Soft Imaging System GmbH, Munster, Germany). The arithmetic mean of the measurements was used in the calculations of tree growth. Latewood width in each ring was also measured, and its proportion in the ring was calculated.

Tree growth at the base and at the 5 m height level was characterized by the mean annual ring width (RW), the mean annual ring for the initial growth corresponding to the first 20 years from pith (IRW) and the mean annual ring for the final growth in the last 10 years before felling (FRW). Two relative growth factors were calculated: relative initial growth (IG) and relative final growth (FG) by dividing the corresponding mean initial and final annual ring width by the mean annual ring (IRW/RW and FRW/RW, respectively).

Statistical and correlation analysis was performed using Microsoft EXCEL 2013 procedures. Regression analysis was characterized by means of the Pearson correlation and coefficient of determination.

Results

Tree radial growth and ring width

The radial growth measured at stem base in trees from Cabreira showed that for approximately the same tree age of 45 years, the mean wood diameter ranged from 95.0 to 58.4 cm, corresponding to an average radial growth rate of 7.1 ± 2.0 mm $year^{-1}$. The trees from Estrela for an approximate tree age of 50 years had a wood diameter at stem base ranging from 95.4 to 55.4 cm, corresponding to an average radial growth rate of 6.6 ± 1.8 mm $year^{-1}$.

The mean growth rates and the initial (first 20 rings from pith) and final (last 10 rings) growth rates measured at stem base and at 5 m height are reported in Table 2. In both sites, the initial growth rate was similar and also at both height levels, but final growth was considerably higher at Cabreira than at Estrela (8.5 vs 3.7 mm $year^{-1}$ at stem base). The mean growth rate was higher for the trees from Cabreira at the stem base (8.1 vs 5.8 mm $year^{-1}$, respectively). For the trees from Cabreira, the growth rate was more homogeneous at the stem base, i.e. initial and final growth showed similar values relatively to the mean while at 5 m of height the relative initial growth was higher than final growth (1.2 vs 0.8) (Table 2). The growth rates at Estrela were less homogeneous over time, although the absolute and relative growth rates were very similar between the stem base and the 5 m height level. The latewood proportion in the ring was similar at both sites and without differences between base and 5 m of height (Table 2).

In general, the mean ring width profile at the stem base showed an initial slow growth rate up to 5 years of age (mean of 3.9 and 4.5 mm $year^{-1}$, respectively, at Cabreira and Estrela), after which the growth rate increased until about 17 years of age (9.0 and 7.8 mm $year^{-1}$, respectively), decreasing to 7.3 and 5.8 mm $year^{-1}$, respectively, to 45 years of age (Fig. 1).

Heartwood development

Heartwood was present in all the trees up to 25 m of stem height; at the height level of 27.5 m, only two trees from Cabreira contained heartwood.

The vertical development of heartwood was similar in all the trees: the heartwood area decreased from the tree base upwards, and the heartwood diameter followed closely that of the stemwood, also accompanying the butt swelling (Fig. 2).

The proportion of heartwood in the total stem cross section remained stable in the lower part of the stem until 5 m of tree height, after which it decreased steadily to the

Table 2 Tree growth variables and percentage of latewood for Douglas-fir trees (mean of 10 trees ± SD) from two sites (Cabreira and Estrela) measured at stem base and at 5 m of height: RW, mean annual ring width; IRW, mean annual ring for the initial growth in the first 20 years from pith; FRW, mean annual ring for the final growth in the last 10 years before felling; IG, relative initial growth and FG, relative final growth

	Cabreira		Estrela	
	Base	5 m	Base	5 m
Ring width (mm)				
Mean(RW)	8.07 ± 4.04	7.20 ± 2.49	5.75 ± 2.94	5.68 ± 2.67
Initial (IRW)	7.79 ± 4.33	8.51 ± 2.26	7.75 ± 2.85	7.78 ± 2.28
Final (FRW)	8.48 ± 4.78	5.46 ± 1.32	3.74 ± 1.93	3.38 ± 1.30
Relative growth				
Initial (IG)	0.97 ± 0.16	1.18 ± 0.07	1.34 ± 0.18	1.36 ± 0.10
Final (FG)	1.07 ± 0.24	0.76 ± 0.09	0.61 ± 0.13	0.58 ± 0.09
Latewood (%)	30.3 ± 4.7	30.6 ± 8.6	32.1 ± 8.7	29.3 ± 9.9

Tree age (as the number of rings at stem base), overbark diameter at 1.3 m (d.b.h.) and height of the Douglas-fir trees sampled in the two sites of Cabreira and Estrela

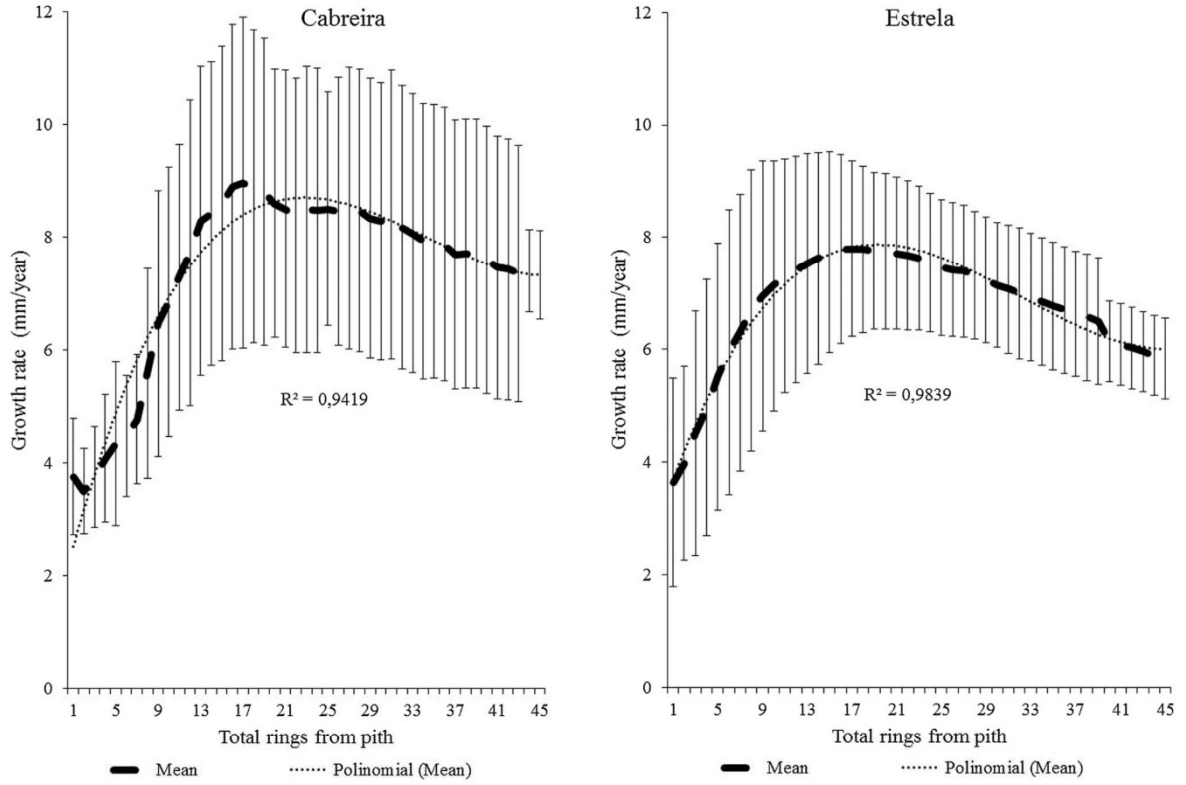

Fig. 1 Mean growth rate (and SD as *bar*) and adjusted polynomial curve, at the stem base level of ten trees from the two sites of Cabreira and Estrela

upper part (Table 3). On average, heartwood in trees from Cabreira and Estrela represented 49% at the base and 5 m height level, 45 and 46%, respectively, between 5 and 10 m of height and decreased to 38 and 40%, and to 25 and 33% for the upper height levels. The heartwood proportion was similar in both sites (Table 3).

The number of growth rings in the heartwood decreased along the stem height with a trend that was similar in all the

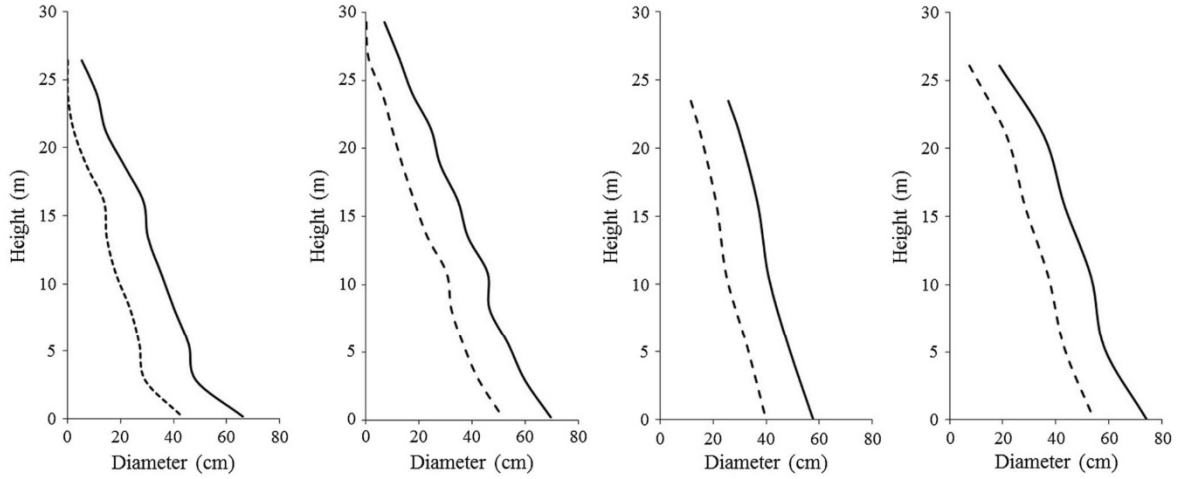

Fig. 2 Vertical profiles of heartwood (*dashed line*) and stem inside bark (*full line*) diameters for four Douglas-fir trees with different butt swelling magnitude (**a**, **b** Cabreira; **c**, **d** Estrela)

Table 3 Variation of heartwood, sapwood and bark proportion (% of the stem cross-sectional area), and of total stem cross-sectional area (cm^2) along the stem for Douglas-fir trees at two sites (Cabreira and Estrela)

Tree height level (m)	Base	5	10	15	20	25
Cabreira						
Stem cross section (cm^2)	3783.4 ± 1250.2	1892.6 ± 287.5	1313.8 ± 338.6	703.6 ± 260.4	288.8 ± 202.3	167.6 ± 104.2
Heartwood (%)	50.4 ± 7.4	48.4 ± 5.2	43.1 ± 6.8	32.6 ± 6.8	17.6 ± 10.6	12.2 ± 4.8
Sapwood (%)	34.9 ± 7.5	43.1 ± 4.9	48.3 ± 6.2	58.7 ± 6.3	73.1 ± 11.4	78.9 ± 5.3
Bark (%)	14.7 ± 3.6	8.4 ± 1.0	8.6 ± 1.3	8.7 ± 1.7	9.3 ± 3.4	10.4 ± 1.4
Estrela						
Stem cross section (cm^2)	3716.6 ± 1334.2	2203.9 ± 422.3	1271.5 ± 360.5	1288.3 ± 383.0	827.2 ± 354.2	555.6 ± 420.7
Heartwood (%)	49.6 ± 5.0	48.1 ± 8.6	42.8 ± 8.0	36.7 ± 8.8	29.2 ± 10.4	18.8 ± 7.1
Sapwood (%)	35.2 ± 5.9	41.8 ± 8.5	48.1 ± 8.0	54.4 ± 8.4	62.9 ± 11.0	73.5 ± 7.3
Bark (%)	15.2 ± 2.1	10.0 ± 1.2	9.0 ± 1.3	8.9 ± 1.4	7.9 ± 0.9	7.7 ± 0.9

Mean of 10 trees ± SD

trees. For example, the 45-year-old tree had 28 heartwood rings at the stem base, 18 at 5 m and 13 at 10 m of height; at the top level, with 6 wood rings, there was no heartwood.

Heartwood formation in relation to age was investigated by considering the variation of the number of heartwood rings (HW) with cambial age (CA) (Fig. 3), and a linear regression could be adjusted with an excellent coefficient of correlation: $HW = 0.8846\ CA - 7.3568$ ($R^2 = 0.991$) for Cabreira and $HW = 0.7280\ CA - 6.7368$ ($R^2 = 0.958$) for Estrela. The slope of the regression line indicated a rate of heartwood formation of an average of 0.9 and 0.7 rings $year^{-1}$, respectively, in Cabreira and Estrela, and heartwood formation was estimated to start at a cambial age of 8 years in Cabreira and 9 years in Estrela. The proportion of heartwood in the stemwood section varied with cambial age, increasing from approximately 12 and 19% for a cambial age of 11 and 21 years, respectively, to 50% for 46 and 50 years for Cabreira and Estrela (Fig. 4).

The average tree (outside bark) and stemwood (inside bark) volumes up to the commercial height level were 2.9 and 2.6 m^3, respectively, for the trees from Cabreira, and 4.2 and 3.7 m^3, respectively, for trees from Estrela. The heartwood volume was 1.3 m^3, corresponding to 44% of the average stemwood volume for the trees from Cabreira and 1.7 m^3, corresponding to 42% of the average stemwood volume for the trees from Estrela.

Sapwood variation

The sapwood cone started above the 25 m height level, thereby representing a length to the top of the tree under

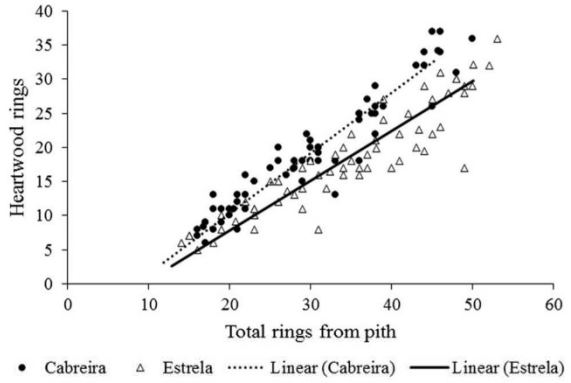

Fig. 3 Variation of the number of heartwood rings as a function of total rings from pith and fitted linear regression curve (Cabreira: HW = 0.8846 CA − 7.3568, $R^2 = 0.991$, and Estrela: HW = 0.7280 CA − 6.7368, $R^2 = 0.958$, with *HW* heartwood rings, *CA* cambial age)

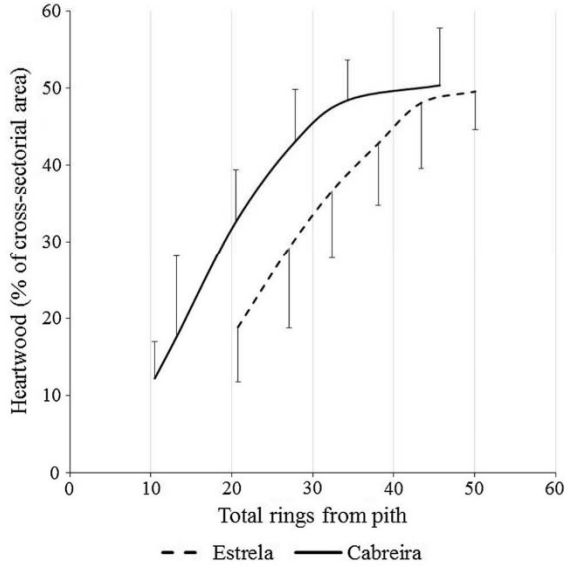

Fig. 4 Variation of heartwood proportion in the stem cross section with total rings from pith for 10 trees from Cabreira and Estrela

approximately 4 and 9 m, respectively, for Cabreira and Estrela. The radial width of sapwood was higher at the base in the region of butt swelling, where it represented on average 75 and 74 mm for Cabreira and Estrela, respectively. Between 5 and 10 m, sapwood width was approximately constant at 62 mm and decreased upwards to 44 mm at 25 m for the trees from Cabreira; for the trees from Estrela, the axial decrease in sapwood width was less pronounced and was 61 mm at 25 m of height (Fig. 5). The proportion of sapwood in the cross section increased to the upper part of the stem (Table 3).

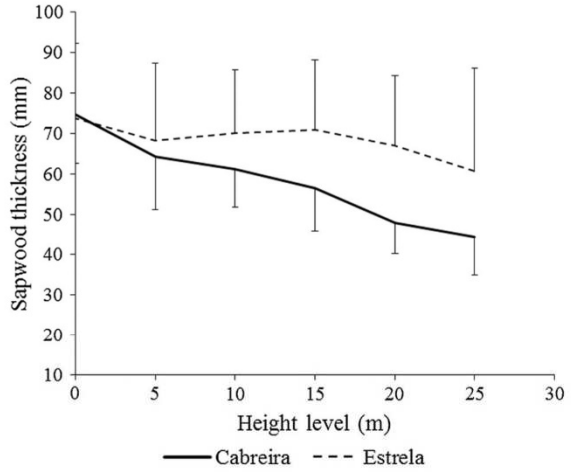

Fig. 5 Variation of sapwood radial width along the tree height in Douglas-fir trees in two locations (Cabreira and Estrela). Mean of 10 trees and SD

Bark development

Within the tree, the mean bark thickness decreased from 26.4 and 26.9 mm at the base to 3.3 and 4.7 mm at the top of the trees, respectively, for Cabreira and Estrela (Fig. 6). Bark thickness could be modelled for these 45- and 50-year-old trees (Cabreira and Estrela) as a function of the tree height level, or of the tree diameter or cambial age with high coefficients of determination:

- Cabreira:
  BT = −4.35 ln(TH) + 18.444 ($R^2 = 0.987$, R^2 adj = 0.984);
  BT = 0.0001 D^2 − 0.0664 D + 15.34 ($R^2 = 0.992$, R^2

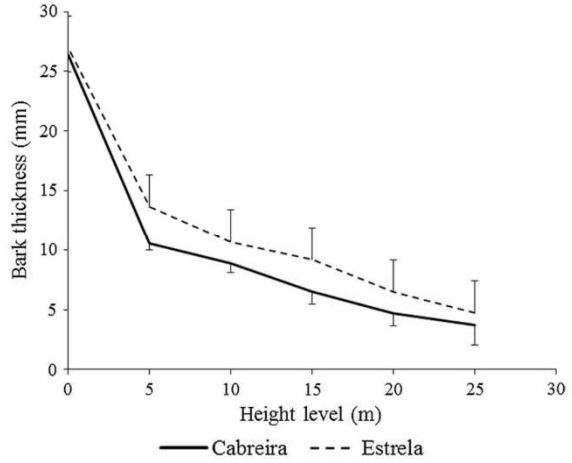

Fig. 6 Axial variation of bark thickness for Douglas-fir trees in two sites (Cabreira and Estrela). Mean of ten trees and SD (as *bar*)

adj = 0.986);
BT = 0.0022 CA^3 − 0.176 CA^2 + 4.7654 CA − 35.546 (R^2 = 0.996, R^2 adj = 0.986).

- Estrela:
  BT = −3.77 ln(TH) + 18.61 (R^2 = 0.975, R^2 adj = 0.968);
  BT = 0.0001 D^2 − 0.0514 D + 11.305 (R^2 = 0.992, R^2 adj = 0.987);
  BT = 0.0021 CA^3 − 0.1972 CA^2 + 6.2184 CA − 58.821 (R^2 = 0.992, R^2 adj = 0.983).

where BT is bark thickness (mm), TH the tree height level (m), D the diameter (cm) and CA the cambial age.

Bark proportion varied along the tree with height (Table 3). The pattern was similar for all the trees, with a higher content in the lower part of the stem (15% at base level) decreasing and stabilizing at 9.0 and 8.0% up to 25 m of height, respectively, for Cabreira and Estrela.

The average bark volume was 0.33 and 0.52 m^3 corresponding to 11 and 12% of the total tree stem volume for Cabreira and Estrela.

Taper

Stemwood taper and heartwood taper for the different logs are summarized in Table 4. At Cabreira, the taper was highest for the butt log reflecting the butt swelling. For the 5–10 m height section, stemwood and heartwood tapers were smaller and similar at an average of 14.9 mm m^{-1}; from 10 m to the upper part of the tree, the stemwood and heartwood tapers were constant at 20.5 and 18.2 mm m^{-1}, respectively (Table 4). For the trees at Estrela, the stemwood taper and heartwood tapers were smaller than for the trees at Cabreira (Table 4).

Discussion

Radial growth and ring analysis

The Douglas-fir trees showed a high radial growth in the two stands in northern and central Portugal, with an average of 6.9 mm $year^{-1}$ (Table 2) and a decreasing pattern with cambial age (Fig. 1). These results are similar to the 7.2 mm $year^{-1}$ of Douglas-fir trees growing in the coastal range of western Oregon (USA) that also showed a rapid early growth and then a gradual growth rate decline (Tappeiner et al. 1997). For Douglas-fir stands with a similar initial tree density of 1200 trees ha^{-1}, Hein et al. (2008) showed that the mean radial increment was below 8 mm decreasing steadily between the start of the experiment until the end of observation.

Growth in the juvenile phase was higher than in mature periods (Table 2), and the difference was more accentuated in the slower growth stand of Estrela; the maximum growth rate was attained at approximately 20 years (Fig. 1), in accordance with previous observations (Louro and Cabrita 1989).

Growth was somewhat higher at Cabreira than at Estrela (Fig. 1), which may be caused by the different site elevations (850 vs 930 m) since growth rate generally slows down with increasing elevation (Lassen and Okkonen 1969). Climate effects of temperature and rainfall were considered in the analysis of ring width of the trees in Cabreira and Estrela, but no significant signals were found (data not shown). Since tree competition is a factor influencing diameter growth (Kohnle et al. 2012), the fact that between-tree competition was probably higher in the regular and pure stand of Estrela may also account for this difference in growth, especially regarding the late growth in the mature trees (Table 2). On the contrary, tree height is only marginally affected by inter-tree competition and may be used as a stand-level indicator for the growth potential of a specific site (Kohnle et al. 2012); in the present context, the tree height was in fact similar for both sites.

This high radial growth in conjunction with tree height (on average 32 m, Table 1) allows a harvest age for the sawmilling industry in the range of 45–60 years (Fontes et al. 2003a), thereby confirming the good potential of Douglas-fir as a timber species for the mountain areas of Portugal, as also previously suggested (Fontes 2002; Fontes et al. 2003b), namely for afforestations directed towards increasing timber production (Diniz 1969; Fontes 1989, 2002; Freitas 1989; Louro and Cabrita 1989). It is interesting to notice that the radial growth rate of Douglas-fir is substantially higher than that reported for *Pinus*

Table 4 Stemwood and heartwood taper, in mm/m, between different stem height levels (mean of 10 trees ± SD) of Douglas-fir trees in two locations (Cabreira and Estrela)

Height levels (m)	Cabreira		Estrela	
	Stemwood	Heartwood	Stemwood	Heartwood
Base-5	31.2 ± 13.2	28.4 ± 9.8	24.0 ± 13.7	21.8 ± 9.7
5–10	15.3 ± 5.5	14.5 ± 3.7	10.8 ± 2.5	11.6 ± 2.6
10–15	20.3 ± 7.0	18.6 ± 5.9	11.8 ± 6.7	12.1 ± 6.3
15–20	20.6 ± 4.1	18.0 ± 4.5	18.2 ± 8.4	15.1 ± 5.1
20–25	20.7 ± 3.9	18.1 ± 5.2	15.5 ± 12.0	15.7 ± 5.4

pinaster that is the most common timber conifer in Portugal, e.g. in mature trees 1.8 and 2.6 mm year^{-1} (Knapic and Pereira 2005; Vieira et al. 2009) or 4.2 mm year^{-1} in 17-year-old trees (Gaspar et al. 2008).

The fact that ring width pattern was similar at different height levels along the stem, i.e. growth rate at the same cambial age was similar for the different tree ages, contributes to stemwood homogeneity which is an advantage when similar mechanical performance of long wood structural elements is desired. Exceptions were found only in some trees at the stem base where higher heterogeneity was found derived from the presence of butt swelling. The similar ring structure, i.e. latewood proportion was similar (Table 2), also gives a considerable homogeneity to the wood.

Heartwood development

Very few references are found in the literature regarding the heartwood content in Douglas-fir and its within-tree development. The trees sampled here in both sites contained a substantial proportion of heartwood that decreased from tree base upwards (Fig. 2). This axial variation pattern was already observed for 34-year-old Douglas-fir trees (Gartner 2002) and follows the general trend described for all species (Hillis 1987), namely for pines such as *Pinus pinaster* (Pinto et al. 2004; Knapic and Pereira 2005; Esteves et al. 2005), *P. sylvestris* (Bjorklund 1999; Morling and Valinger 1999), *P. canariensis* (Climent et al. 2003), *P. contorta* (Yang and Murchison 1992), *P. banksiana* (Yang et al. 1985) and *P. radiata* (Wilkes 1991). Long and Scott (1981) observed that most of the increase in total cross-sectorial area of the stem below the crown is attributable at the non-conducting heartwood.

The heartwood content increases with tree age, and various authors found evidence that, after a certain initiation age, heartwood is formed at a constant annual ring rate (Hazenberg and Yang 1991; Wilkes 1991; Sellin 1994; Bjorklund 1999; Gjerdrum 2003). This was also found here for Douglas-fir with the number of heartwood rings strongly correlated with cambial age. The rate of heartwood formation of 0.9 and 0.7 rings year^{-1} increased with cambial age, and the values were similar to those registered for other softwoods, e.g. for *Pinus pinaster* 0.5 rings year^{-1} (<55 years) and 0.7 rings year^{-1} (>55 years) (Knapic and Pereira 2005), *P. sylvestris* 0.5, 0.7 and 0.9 rings year^{-1} (<45, 90 and 115 years, respectively) (Bjorklund 1999), and 0.6 and 0.8 rings year^{-1} for cambial ages of 60 years and 220 years, respectively (Gjerdrum 2003) and *Picea mariana* 0.79 rings year^{-1} (50 years) and 0.98 rings year^{-1} (90 years) (Hazenberg and Yang 1991).

Heartwood formation was estimated to start at a cambial age of 8 and 9 years in accordance with works of Smith et al. (1966) who indicated an early formation of heartwood in Douglas-fir. Other species show different ages for heartwood initiation: for *Pinus pinaster* 13 or 18 years (Knapic and Pereira 2005; Esteves et al. 2005), *P. sylvestris* 15–25 years (Bjorklund 1999; Morling and Valinger 1999), *P. canariensis* 30 years (Climent et al. 2003) and *Picea abies* 18–20 years (Munster-Swendsen 1987). These results indicate that heartwood initiation age and formation rate are species specific.

Heartwood formation may also be more dependent on tree diameter than on age, as reported for several species, e.g. *Tectona grandis* (Kokutse et al. 2004), *Acacia melanoxylon* (Knapic et al. 2006), *Eucalyptus globulus* (Gominho and Pereira 2000, 2005) and *Pinus sylvestris* (Bjorklund 1999). In fact, heartwood dimensions have positive variations with tree growth, e.g. *Larix decidua* (Leibundgut 1983) and *Eucalyptus globulus* (Gominho and Pereira 2000), and stem diameter was a good predictor of heartwood diameter in *Pinus pinaster* (Pinto et al. 2004) and *P. canariensis* (Climent et al. 2003).

Sapwood variation

The mean sapwood width of 75 mm was in accordance with Domec et al. (2012) who reported 72 mm of sapwood width for Douglas-fir trees with 61–63 cm d.b.h (100–102 years old). The axial variation of sapwood width was of small magnitude with a decrease from stem base to the top which was more accentuated in Cabreira than in Estrela where it showed an almost constant value of 70 mm (Fig. 5), also reported by Long and Scott (1981) for 45-year-old Douglas-fir trees. This vertical distribution of sapwood supports the pipe model described by Shinozaki et al. (1964) by which the sapwood cross-sectorial area remains more or less constant below the live crown. The near constancy of sapwood width with height in Douglas-fir after initial higher values at stem base was also described for trees of different ages, e.g. 22 years old (Bancalari et al. 1987), 34 years old (Gartner 2002), 55 years old (Megraw 1986) and 59 to 78 years old (Wellwood 1955). This pattern of sapwood width variation along the Douglas-fir trees (Fig. 5) is also in accordance with that reported for other conifers, e.g. *Pinus pinaster* (Stokes and Berthier 2000; Knapic and Pereira 2005; Pinto et al. 2005), *P. sylvestris* (Bjorklund 1999), *P. banksiana* (Yang et al. 1985) and *P. contorta* (Yang and Murchison 1992). Sapwood width generally increases with tree diameter, rate of growth and relative crown size (Lassen and Okkonen 1969).

The most rapidly growing Douglas-fir trees have the widest sapwood (Wellwood 1955; Smith et al. 1966), and sapwood area is positively correlated with tree growth, largely due to its correlation with tree foliage area or mass (Grier and Waring 1974). The live crown height or

diameter was not measured in this present work, and therefore, no further relation to sapwood development may be made. However, in the observed Douglas-fir trees, the radial growth rate was only weakly related to sapwood amount (data not show), and these results agree with others reported previously for Douglas-fir (Lassen and Okkonen 1969).

The number of sapwood rings at stem base (on average 10 rings) is approximately the same reported for 34-year-old Douglas-fir trees but smaller than the 30 and 40 rings observed for 100-year-old trees (Domec et al. 2012). The number of sapwood rings showed a small increase with cambial age up to a maximum at about 35–40 years followed by a decline after this age, therefore corresponding to a within-tree variation with height, in accordance with Bancalari et al. (1987) and Brix and Mitchell (1983). The small within-tree variation of sapwood width results in an increasing sapwood area proportion from stem base (on average 35% of the cross section) to the top (e.g. 57% at 15 m of height) (Table 3), as also observed by Hein et al. (2008). Domec et al. (2012) also documented a sapwood proportion increase from 30 to 53% corresponding to 100 and 34 years, respectively. Hein et al. (2008) also reported that stand density did not have effect on the cross-sectorial sapwood proportion along the stem, with sapwood extent appearing to mirror stem taper quite well.

Taper

In the sampled Douglas-fir trees, the stemwood and the heartwood showed a similar vertical profile (Fig. 2), as already described (Long and Scott 1981), and therefore, the wood and heartwood taper values were similar (Table 4). Taper was higher in the trees from Cabreira in relation to Estrela, e.g. the average taper of the trees from 5 to 25 m of stem was, respectively, 19.2 and 14.1 mm m^{-1}; this may be explained by a stronger tree competition in the pure Estrela stand. Overall, the stems were more cylindrical than would be implied by conformity to a cone, and the taper values were low which stresses the stem quality of Douglas-fir for long wood components.

Bark development

Bark thickness is a phenotypic characteristic important for forest management, the development of underbark timber volume equations, and may be associated with fire resistance (Kohnle et al. 2012). The latter aspect is particular important for Portugal where forest fires are frequent summer occurrences, and the high cork content in Douglas-fir bark confers an added insulation potential (Ferreira et al. 2015, 2016). Bark represented on average 12% of the stem volume, as also observed by McConnon (2004) and lower than the 18% referred by Maguire and Hann (1990). Bark showed, however, a significant thickness variation from the tree base upwards (Table 3; Fig. 6), as also reported by Gartner (2002). Bark thickness of Douglas-fir trees at any point above breast height can be estimated by a segmented polynomial taper equation given total height and breast height diameter (Maguire and Hann 1990).

Bark development in trees is a cumulative process, and therefore, the decrease in bark thickness with tree height is a common feature on several species (Trockenbrodt 1994), e.g. *Eucalyptus grandis* (Wilkins 1991) and *E. globulus* (Quilhó et al. 2000). Since bark of Douglas-fir may be a significant raw material if addressing a full resource use and a biorefinery approach (Ferreira et al. 2015, 2016), the results suggest that the debarking of the butt logs up to 5 m of height (where bark thickness is highest) would be the most efficient.

Conclusion

Douglas-fir showed good potential as a timber species for the mountain areas of Portugal with high radial growth that increased with age to a maximum growth rate at approximately 20 years.

The Douglas-fir trees contain a substantial proportion of heartwood estimated to start early at 8–9 years of cambial age and increasing by 0.7–0.9 rings $year^{-1}$. Sapwood width was on average 75 mm with small axial variation. The bark content is high especially in the lower part of the stem.

The stems showed a high axial homogeneity as regards ring development and low taper confirming their aptitude for long wood components for structural construction applications.

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Cork of Douglas-fir bark: impact of structural and anatomical features on usage

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Cork of Douglas-fir bark: Impact of structural and anatomical features on usage

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ABSTRACT

Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) is one of the best conifer timber species. Its bark contains a substantial proportion of cork that may have a valorization potential, given adequate structural and cellular features. This study was made here on

bark samples from mature trees from the north and central mountains of Portugal. The area proportion of cork was determined by image analysis, the cork tissue was observed with electron scanning microscopy (SEM) and cell dimensions measured.

The cork is not continuous within the rhytidome, and the layers are interspersed with phloem regions. The cork layers are not continuous along the tangential or axial directions. Older trees contain on average a thicker rhytidome and a higher proportion of cork. The cork tissue was characterized by the presence of extensive areas of cells that are crushed or completely collapsed, making up a compressed and very compact structure with patches of uncompressed cork. The compression occurs in the radial direction and is clearly observed in transverse and radial sections. In the uncompressed regions the majority of the cork cells are hexagonal and pentagonal prisms stacked base-to-base and aligned in the radial direction in parallel rows. On average, the prism height and base area are 55 μm and 1388 μm^2, respectively, with a 1.3 μm cell wall thickness. To obtain pure cork fractions from Douglas-fir bark, trituration and fractionation processes are needed. Also the use of Douglas-fir cork as a cellular material will be restricted by the extensive cell compression.

1. Introduction

The cork that is stripped from the outer bark of the cork oak (*Quercus suber* L.) is one important European non-wood forest product and an economically relevant industrial raw material. The cork oak forests are geographically restricted to the western part of the Mediterranean with the largest production concentrated in Portugal and Spain (Pereira and Tomé, 2004). Cork has a closed cellular structure which together with the chemical features are the basis for its specific combination of properties: for instance low density, permeability and heat transfer, with large compressibility without fracture, and high durability (Pereira, 2015). The multiple applications of cork are recognized around the world: wine stoppers, cork composite materials e.g. insulation boards, surfacing materials, and other products (Pereira, 2007).

There are other tree species with barks that contain substantial amounts cork and some were characterized as potential cork providers: *Quercus cerris* (Turkey oak) (Şen et al., 2011a), *Quercus variabilis* (oriental or Chinese cork oak) (Miranda et al., 2013b), *Betula pendula* (Pinto et al., 2009) and *Kielmeyera coriacea* (Rios, 2011). The utilization of cork from the bark of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) was also considered triggered by the large availability of this bark, namely in northern America (Hergert and Kurth, 1952; Krahmer and Wellons, 1973; Litvay and Krahmer, 1977).

Douglas-fir is one of the best timber conifer species, autochthonous to North America, and now also widely distributed in European forests: it occupies approximately 19 million ha in the USA and Canada (Weiskittel et al., 2012) and over 550 thousand ha in Europe. Douglas-fir provides knot-free sawn timber of great length and quality due to the large stem size and excellent wood properties. Most timber comes from plantation forests in Europe and North America (Ross and Krahmer, 1971).

Large quantities of Douglas-fir bark are therefore available as a residue from the primary processing of logs at sawmills or other processing mills. The potential of Douglas-fir bark was recognized

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early and several studies were published, as compiled by Kurth (1950) and Hall (1971). One of the interesting features of Douglas-fir bark is that it contains a substantial proportion of cork in the rhytidome (Krahmer and Wellons, 1973; Patel, 1975).

The idea of commercially using the cork component of Douglas-fir bark is not new and was proposed in 1943 by Professor Bror Grondal (University of Washington) when cork was considered a vital war product, therefore potentially allowing the U.S. to become self-sufficient ("Eugene Register-Guard – Google News Archive Search," 1943). However this did not turn into reality even if some studies were continued (Laver and Fang, 1989; Graça and Pereira, 1999).

Douglas-fir bark structure is complex and cork is present in a substantial proportion. However the cork is not continuous within the rhytidome, and the layers are interspersed with phloem regions which makes cork separation difficult (Ferreira et al., 2015a). This is what occurs also in other cork containing barks with a similar rhytidome architecture, as for instance *Q. cerris* (Şen et al., 2011a). Although the structure of bark and cork of Douglas-fir was already described (Chang, 1954; Grillos, 1956; Hall, 1971; Hergert and Kurth, 1952; Litvay, 1976; Patel, 1975; Percival, 1948; Ross and Krahmer, 1971), it was not analyzed in detail from a material's point of view, namely regarding the cellular features of cork that would impact on its properties and product performance (Pereira, 2015).

This is the aim of the present study where the structure of the cork tissue in the bark of Douglas-fir was described in relation to topological arrangement, geometry and dimensions of the cells, and discussed regarding impact on properties and potential uses. A comparison is made with the cellular characteristics of cork from *Q. suber* which is the benchmark for this type of material. The objective is to contribute for the potential utilization of Douglas-fir bark residues through the valorization of its cork component.

2. Materials and methods

Bark samples of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) were collected from a total of 20 trees in two sets (ten trees each) with approximately 44 and 100 years of age, respectively in the north and central mountains of Serra da Cabreira (41°35′18.0″N, 8°01′00.6″W) and Serra da Estrela (40°19′18.5″N, 7°36′49.8″W) in Portugal. The bark samples were collected from the bottom part of the trees, from the base up to 1.3 m of stem height. The bark samples were stored in indoor conditions with low light and good ventilation.

The transverse sections of the bark rhytidome were observed by image analysis and the area proportion of cork was calculated.

For electron scanning microscope (SEM) observations, small cubes with approximately 3 mm of edge were cut with a sharp razor blade in the cork region within the rhytidome. The cubes were mounted on stubs (ProSciTech, Australia) and sputter coated (Polaron E 5100 E, USA) with gold palladium for 3 min at 20 mA. The transverse, tangential and radial sections surfaces were observed in an SEM Hitachi S-2400 at magnifications ranging from 50 to 1000×, and the images were recorded in digital format.

In the SEM images corresponding to the different sections (transversal, tangential and radial) the cell measurements were made using an image analysis software (Leica Qwin Plus). The measurements were averaged for the tangential section (the honeycomb type cellular arrangement) and the non-tangential sections (the brick-wall type of structure) (Fig. 1). This type of measurements was only possible in the tissue regions where the cells were not corrugated or crushed, therefore representing the potential non-compressed cellular tissue. In the corrugated or crushed regions of cork, the measurements of individual cells cannot be made and only descriptive observations and general structural features are given.

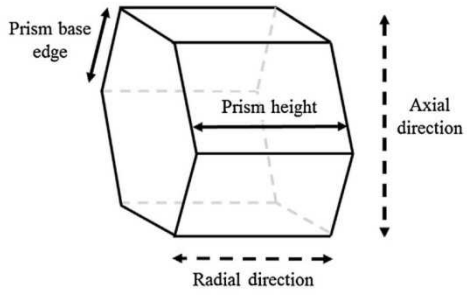

Fig. 1. Schematic drawing of a cork cell as a hexagonal prism showing the axial and radial directions and indicating where prism base edge and prism height were measured.

The number of edges of each cell i.e. the number of neighboring cells, was counted on the tangential and non-tangential sections. The distribution function of the number of edges of each cell was calculated based on the results of a total of 400 cells for each section as $f_i = N_i/\sum N_i$ where Ni represents the number of cells with i edges and $\sum N_i$ the total number of cells. The topological disorder of the bi-dimensional networks was evaluated by the dispersion of the function in relation to the mean (im) was calculated as $\mu_2 = \sum (i - i_m)^2 f_i$.

The average cell area was measured on the tangential sections, corresponding to the average prism base area, and the cell prism height was measured on the non-tangential sections. The cell wall thickness was measured in tangential and non-tangential sections as the radial dimension (Fig. 1). A total of 400 cells were measured for each section.

Fractionation of the Douglas fir rhytidome was made by using a cutting mill (RetschSM 2000) and a first pass with an output sieve with 10 mm × 10 mm openings, and a second pass with 6 mm × 6 mm openings, and sieved with a vibratory sieving apparatus (Retsch AS 200 basic) with standard sieves with mesh sizes of 80 (0.180 mm), 60 (0.250 mm), 40 (0.425 mm) and 20 (0.850 mm). The mass retained on each sieve was weighed and the corresponding yields were determined. Water flotation was applied to obtain cork fractions with higher purity after the laboratory scale fractioning. The fraction retained in the 20 mesh sieve was separated by flotation in distilled water with 24 h settling time after an initial mixing into a floating fraction of cork-enriched granules and a submerging fraction of phloem-enriched granules. Both fractions were separated, dried and weighed. Two replicates were made for the fractionation experiments.

3. Results

3.1. Rhytidome structure

The rhytidome of Douglas-fir bark comprises bands of phloem alternated with cork tissues visible to the naked eye: the cork has a light cream brown color and the phloem are dark brown (Fig. 2). The regions of cork are in general disposed as successive layers along the tree circumference but they are not continuous along the tangential or axial directions, and form a patchy pattern in the three sections. An estimate done by image analysis area measurement in the transverse section of bark pieces led to an average cork area proportion of 58% and 49% respectively in trees with 100 and 44 years of age; the older trees contained on average a higher proportion of cork although the range of variation was high. The radial width of the cork layers was also highly variable from about 1–4 mm.

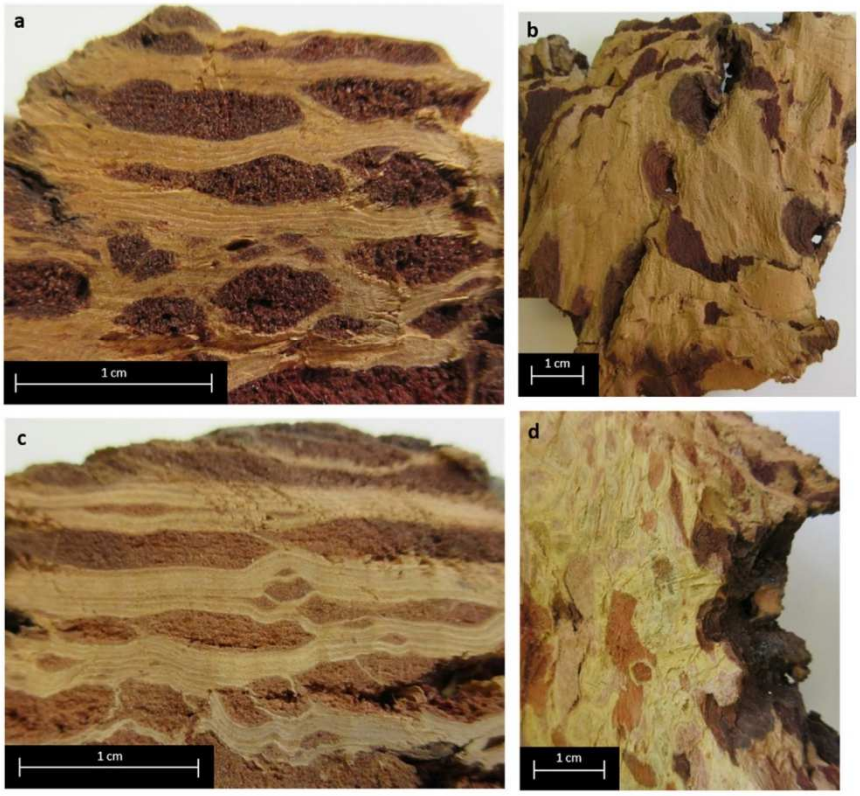

Fig. 2. Rhytidome of Douglas-fir bark. a) transverse section from a 100-yr-old tree; b) tangential section from a 100-yr-old tree; c) transverse section from a 44-yr-old tree; d) tangential section from a 44-yr-old tree.

In the bands of cork tissue a layering could be visually observed with parallel layers of lighter and darker color forming successive growth increments (Fig. 2a and c).

The rhytidome fractioning resulted in the following fraction yields: particles over 20 mesh 56.3%, 20–40 mesh 19.6%, 40–60 mesh 9.6%, 60–80 mesh 4.9% and below 80 mesh 9.6%. The macroscopic appearance of the fractions was very different and a visual observation showed that the coarser fraction (>20 mesh) was enriched in cork while the finer fractions were mostly constituted of phloemic particles. The water flotation yielded a floating layer corresponding to 70.2% of the coarse fraction.

3.2. Cork structure

The structural features of cork in the rhytidome of Douglas-fir bark were observed by scanning electron microscopy in the three principal sections. The striking aspect is that the cork tissue contains extensive areas of cells that are crushed or very heavily corrugated, making up a compressed and very compact structure; these areas border layers, or include patches, of uncompressed cork with the cells showing the cork typical arrangement, without distortion or heavy cell wall undulation. Fig. 3 shows examples of such features.

The compression, and the resulting cell wall folding, occurs in the radial direction and the effect was therefore clearly observed in the transverse and radial sections (Fig. 3a and b). Different intensities of cell collapse occur in nearby regions (Fig. 3c and d): in some areas, the cells are completely collapsed, and only a succession of cell walls is seen, with very little empty spaces between them; in others, the cell walls are corrugated to an high extent, with considerable cell distortion, resulting in a much reduced cell lumen area; cells with little wall undulations also occur interspersed in the tissue.

Overall the areas of compressed cells overweighed the regions with uncompressed cells without or with only light cell wall undulations that occurred in more limited extent. The general pattern observed in the transverse and radial sections was therefore of a more or less tangentially layered structure with compact bands of compressed cells separated by bands of less compressed cells (Figs. 2 and 3). Although very variable between samples. the compacted regions of cork represented approximately 60% of the total cork tissue.

3.3. Cell topology and dimensions

In the uncompressed regions, the cork cells appear in the tangential section as polygons with a honeycomb-type structure and in the non-tangential sections (radial and transverse) as a brick wall-type with a radial alignment in parallel rows (Fig. 4). There were no voids between cells. Cell measurements were made only in such uncompressed areas since in the compacted cork regions it is impossible to make that measurement in individual cells, namely the prism height that depends on the degree of cell compression (Fig. 3).

The majority of the cork cells have six and five sides: in the tangential section 51% of the cells have six edges and in non-tangential sections 69% have five sides (Table 1). The dispersion around the average of the number of polygonal edges is low. Therefore the cork tissue in Douglas-fir bark may be described as consisting of cells that are on average hexagonal prisms stacked base-to-base

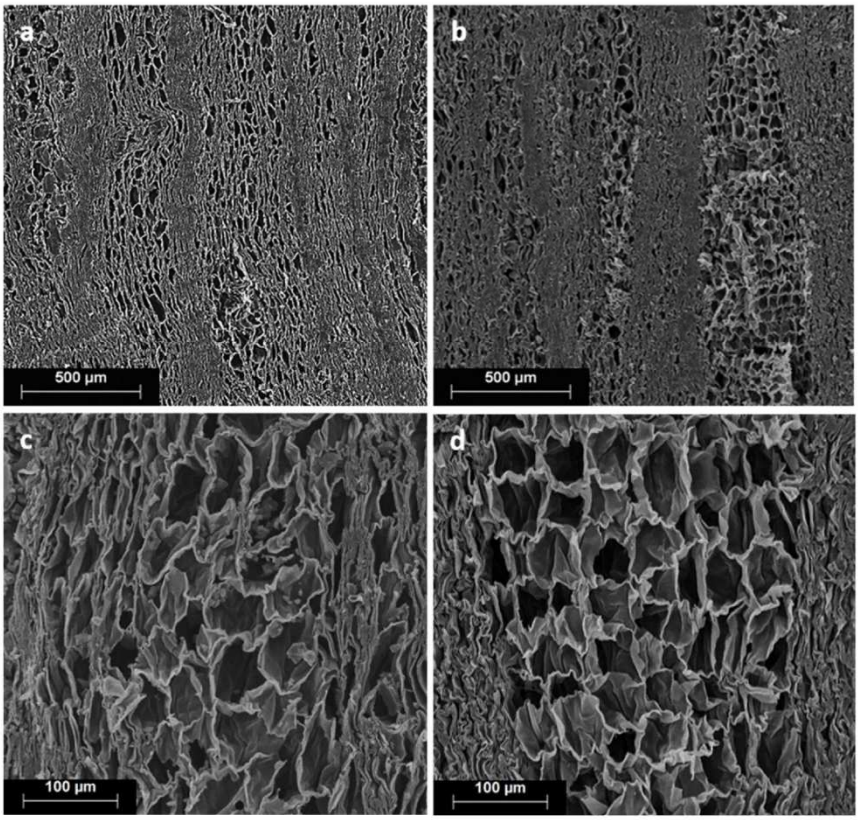

Fig. 3. Cork in the rhytidome of Douglas-fir bark, showing the heavily compressed regions with areas of uncompressed cell tissue in transverse section (a, c) and in radial section (b, d).

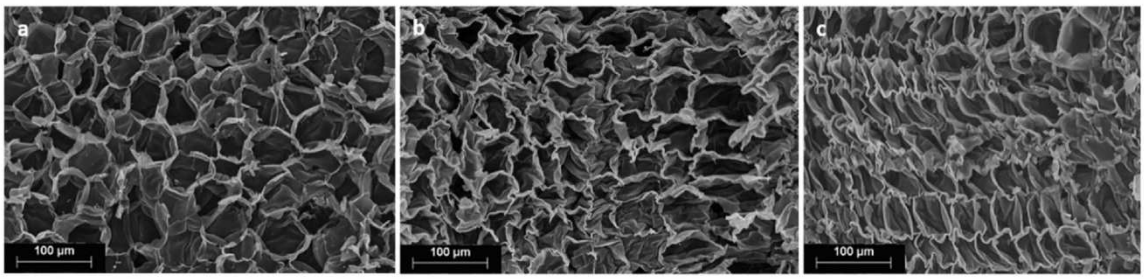

Fig. 4. Tangential (a), radial (b) and transversal (c) sections of cork of Douglas-fir showing the compression of the cells.

and aligned in the radial direction in parallel rows. The transverse and radial surfaces are essentially identical (Fig. 4).

There was variation of cell size along the radial direction, but the cell collapse did not allow to distinguish cork growth rings or the occurrence of late cork cells. In fact a large proportion of cells are extremely compressed forming a compact mass of cells (Fig. 3).

Table 1
Frequency distribution of the number of edges of cells in the tangential and non-tangential sections of cork from Douglas-fir.

Number of edges	Tangential section	Non-tangential sections
4	0.000	0.011
5	0.307	0.685
6	0.505	0.303
7	0.143	0.000
μ^2	0.423	0.229

The average cell dimensions are summarized in Table 2. On average, the prism height (radial dimension) was 55 µm, the prism base area was 1388 μm^2 corresponding to a base edge of 25 µm, and the cell wall thickness 1.3 µm.

The cell wall undulations occur mainly in the radially aligned cell walls i.e. the lateral faces of the prism, with the bases showing only bending except when appreciable distortion is present. The significant extent of undulation results in a crisscrossing pattern of wrinkles, that are often sharp (Fig. 5). No cell wall fracture was observed namely in the heavily wrinkled walls.

Many of the cork cells showed abundant deposits in the lumen and on the inside cell wall that were visible both in the uncompressed cells as well as in the crushed cellular regions (Fig. 6b and

Table 2
Cellular dimensional characteristics of cork cells from Douglas-fir measured on tangential and non-tangential sections (radial and transverse) in the areas without compressed cells. Mean (N = 400) and standard deviation (in parenthesis). For comparison the corresponding values for Q. suber cork (early cork are included ().

	Douglas fir	*Quercus suber*
Prism height (μm)	55.4 (13.9)	30–40
Prism base edge (μm)	24.7 (4.7)	13–15
Average base area (cm^2)	13.9×10^{-6}	$4–6 \times 10^{-6}$
Radial cell wall thickness (μm)	1.3 (0.3)	1–1.5
Tangential cell wall thickness (μm)	1.2 (0.4)	1–1.5
Total cell volume (cm^3)	3.4×10^{-8}	$1–3 \times 10^{-8}$
Number of cells per cm^3	2.9×10^{7}	$4–7 \times 10^{7}$
Solid volume fraction (%)	7.6	8–9

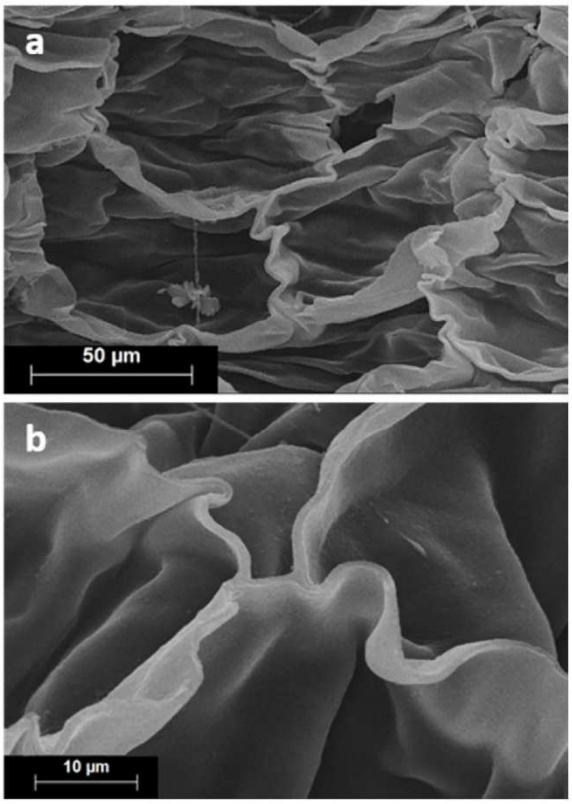

Fig. 5. Transverse sections of Douglas-fir cork showing cell wall undulations mainly in the radially aligned cell walls.

c). These deposits included a granular type of material as well as prismatic needles clusters (Fig. 6a).

4. Discussion

The structure of the rhytidome of Douglas-fir bark with successive periderms alternated by bands of phloem, and containing conspicuously visible cork areas was quite similar to that described for *Quercus cerris* var. *cerris* from Turkey (Şen et al., 2011a). The proportion of cork is high, as it has been reported previously e.g. 35% (Ross and Krahmer, 1971) to 50% (Percival, 1948).

Coniferous barks normally have only a small quantity of cork e.g. *Pinus pinaster* (Nunes et al., 1996), *P. pinea* (Nunes et al., 1999), *P. radiata* (Sands, 1975), but cork is comparably more abundant in the barks of Abies and Pseudotsuga than in other genera (Chang, 1954).

The structural features of the Douglas-fir rhytidome have implications in its potential use. From one side, the substantial proportion of cork in its bark confirms its interest as a cork-provider species, but on the other side the small dimensions of the cork layers (e.g. the radial thickness of the cork layers was <4 mm) and its discontinuous distribution with interspersed phloem tissues (Fig. 2) restrict its use and require a separation procedure. Trituration and fractionation will be required to separate a cork fraction (Ferreira et al., 2015a). Therefore the cork will be obtained in the form of particulate material (cork granules) that can be used only in agglomerated form e.g. in cork agglomerates or in association with other materials in composite agglomerates.

Although Krahmer and Wellons (1973) referred cork layers in Douglas-fir up to 1.2 cm radial thickness with occasional very thick cork layers near the root collar, other measurements referred much lower values of 1.8 mm for the radial thickness of the cork layers at stem base (Ross and Krahmer, 1971). Therefore the structural arrangement of the Douglas-fir rhytidome leads to the need of a cork granulation process and its availability for applications only as cork granules that can be further processed into agglomerates. The trituration of Douglas fir rhytidome and separation of the granulates by particle size was selective by enriching the coarse fraction in cork granules; this is because cork does not fracture under compression and allows large deformations (Pereira, 2007, 2015), while the lignocellulosic phloem regions are brittle and fracture more easily. It is known that bark structure influences trituration and particle size distribution (Baptista et al., 2013; Miranda et al., 2012, 2013a). This was also shown for trituration of Douglas fir rhytidome with a cork enrichment in the coarse fractions (Ferreira et al., 2015a) and can be further refined by densimetric separation or by water flotation, as it was done in the present work. It was possible to obtain a cork fraction representing 39.4% in mass of the rhytidome, which matches the visual observation of the area percent of the cork layers.

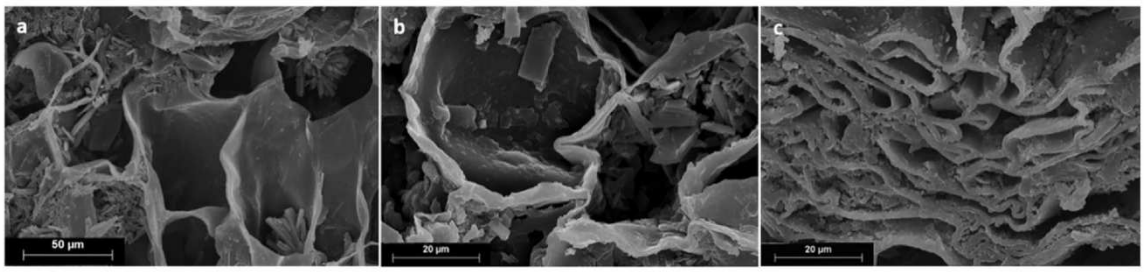

Fig. 6. Deposits in the lumen of cork cells in the rhytidome of Douglas-fir bark showing different type of materials that are deposited in uncompressed (a, b) and compressed (c) cells.

This is clearly different from what happens with Quercus suber where a continuous, thick and homogeneous cork layer is produced that can be processed into solid natural cork products such as the wine stoppers (Pereira, 2007).

The striking feature of Douglas-fir cork is the major presence of bands of compressed cork cells that build up a compact layer of crushed cells (Fig. 3). The densification results from a severe folding of the radial cell walls and the crushing of cells, as it was already previously referred (Ross and Krahmer, 1971). These bands of high solid content appear as dark layers, with a successive tangential alignment, separated by light colored layers (Fig. 2). The calling of these layers as growth increments was tempting, as it has been done in the past (Chang, 1954; Krahmer and Wellons, 1973; Ross and Krahmer, 1971), even as annual increments (Grondal 1942 cit in Ross and Krahmer, 1971). In fact this is the case in commercial cork where annual growth rings are visible and there is a distinction of the earlycork cells (produced in spring and summer) and the darker latecork region (with cells produced in autumn) (Pereira et al., 1987). Bands of more corrugated cells are also found in commercial cork but crushed cells only appear very occasionally and do not include more than 2–3 cells in a radial row. In the case of Douglas-fir, the cork layering in fact included wider bands of dark color and thinner bands of the light colored tissue (Fig. 2).

It is true that cork cells have the capacity to be compressed by folding of the cells walls when they are subjected to a compressive stress: the stress-strain curves of cork show a first elastic region corresponding to cell wall bending, followed by a long plateau with folding of cell walls and ending with densification with cell compaction without fracture (Anjos et al., 2008). This is one of the most interesting properties of cork that results from the structural characteristics and the chemical features of the cell wall (Pereira, 2015).

The densified layers shown by Douglas-fir cork are the result of compressive stress along the radial direction from inside outwards, caused by the tree diameter growth. The cork cells are compressed against the hard, heavily lignified phloem layers that separate the successive periderms, and therefore constitute a rigid barrier e.g. lignin content in phloem is 35% and 17% in cork of Douglas-fir rhytidome (Ferreira et al., 2015b). The high suberin content in the cell walls of Douglas-fir cork (51% of the structural compounds) (Ferreira et al., 2015b) explains that the cell wall may corrugate without fracture (Fig. 5). The question of the number of growth years included in each periderm (i.e. counted in its cork layer) remains therefore an open question.

The high content of crushed cells in the cork of Douglas-fir (of approximately 60%) severely conditions its properties, namely those for which cork is valued as a material and that are the consequence of a cellular structure: low density, low heat transfer, acoustic insulation and vibrational absorption. In fact a regular structure with small cells, as shown in the uncompressed cork regions (Figs. 3 and 4) is required to have such properties. The possibility of making a cell expansion by the unfolding of the radial walls of the cork cells has been reported (Krahmer and Wellons, 1973) and was preliminarily confirmed by us (data not shown). In this case it would be possible to restore, at least partially, a cellular structure for the total extent of the cork tissue. This is certainly a matter that will determine the usage aptitude of Douglas-fir cork.

The cellular description (Fig. 4) and biometry (Table 2) of Douglas-fir cork was therefore made in the uncompressed regions. In general terms it matches the previous reports (Krahmer and Wellons, 1973; Ross and Krahmer, 1971). The cellular structure of Douglas-fir cork is similar to that found in corks of other species as in *Q. suber* (Pereira et al., 1987), *Q. cerris* (Şen et al., 2011b), *Q. variabilis* (Miranda et al., 2013b) and *Kielmeyera coriacea* (Rios, 2011). Some differences are, however, present as discussed below.

The cells of Douglas-fir cork (Table 2) are larger than those described for other species: the prism height is 55 μm in comparison to 30–40 μm in *Q. suber* (Pereira, 2007), 25 μm in *Q. cerris* (Şen et al., 2011a) and 21 μm in *Q. variabilis* (Miranda et al., 2013b), while the base edge is 25 μm and compares to the 13–15 μm, 16 μm and 17 μm, respectively. The cell wall thickness is similar to that reported for *Q. suber* (Pereira, 2007) and *Q. variabilis* (Miranda et al., 2013b) in earlycork cells (1–1.5 μm). Consequently Douglas-fir cork cells have a larger cell volume (3.4×10^{-8} cm^3) and less number of cells per unit volume in comparison with *Q. suber* cork.

The fraction of solid material in Douglas-fir cork (8%, Table 2) is slightly lower than the 10% reported for *Q. suber* cork (Pereira, 2007) and much below that for *Q. cerris* (22%) (Şen et al., 2011a) and *Q. variabilis* (13%) (Miranda et al., 2013b). In consequence, the uncompressed cells of Douglas-fir cork will have a competitive low density in relation to *Q. suber* cork which is advantageous e.g. for insulation purposes.

The topology of Douglas-fir cork cells has a low dispersion in the tangential and non-tangential sections (Table 1). While most polygons in the tangential section are 6-sided with a substantial proportion of 5-sided cells, as previously reported (Krahmer and Wellons, 1973), in the non-tangential section the large majority of cells have 5 sides. This differs from *Q. suber* cork where the three sections are topologically very similar (Pereira et al., 1987).

The biometric and topological features of cork from Douglas-fir e.g. large cells with thin walls, contribute to lower resistance to radial compression and the appearance of the extensive bands with crushed cells, as discussed previously. This explains the difference in comparison with *Q. cerris* that has a similar rhytidome structure with successive periderms but whose smaller and thicker cells show no substantial compaction (Şen et al., 2011a).

Latecork cells were not visible in the cork of Douglas-fir, as described for *Q. suber* (Pereira, 2007), *Q. cerris* (Şen et al., 2011a) and *Q. variabilis* (Miranda et al., 2013b) although it cannot be excluded that they are present, for instance in the compacted cell bands and therefore not visible.

Also a striking feature of the cork cells of Douglas-fir is the presence of conspicuous deposits on the lumens (Fig. 6). This is in accordance with the high content of extractives which represent 29% of cork including a substantial amount of polar compounds soluble in ethanol and water (Ferreira et al., 2015b). This differs from the cork of *Q. suber* with 16% extractives (Pereira, 2013). Again this feature has to be taken into account, namely if a use as liquid sealant is envisaged e.g. in agglomerated cork stoppers for wine bottles, since some of the extractives may be solubilized into the liquid.

5. Conclusions

The rhytidome of Douglas-fir bark has a substantial amount of cork that appear as thin layers that are spatially discontinuous, and interspersed by phloem. The utilization of the cork material will require a trituration process of the bark and a fractionation in order to separate pure cork fractions. Therefore cork will be obtained from the Douglas-fir bark only as a granulated material that can be processed into agglomerates.

The striking feature of Douglas-fir cork is the presence of massive bands of crushed or heavily corrugated cells that are radially compressed, making up a compressed and very compact structure with patches of uncompressed cork. The use of Douglas-fir cork as a cellular material with properties similar to those of commercial cork from *Q. suber* is therefore not possible without cell expansion and straightening of cell walls. The uncompressed cork cells of Douglas-fir are larger and thinner walled than corks from other species, therefore with a lower solid volume fraction which may be a competitive asset in light-weight insulation applications.

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Influence of cambial age on the bark structure of Douglas-fir

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ORIGINAL

Influence of cambial age on the bark structure of Douglas-fir

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Abstract

Douglas-fir (*Pseudotsuga menziesii*) is a valuable conifer timber species. It has a thick bark with a high proportion of cork in the rhytidome that allows considering its recovery. This study focuses on the characterization of the bark features and their variation with cambial age along the stem using samples of 20 trees from two sites in Portugal at harvest for the sawmilling industry. The morphology and anatomical features of bark were examined including a detailed analysis of the arrangement of tissues, cell biometry, tissue proportion of the phloem, and the development of the rhytidome. Bark structure varied within the tree with cambial age at various height levels, and differences concerned mostly the rhytidome and periderm development, tissue morphology and disarray in the non-conducting phloem. A relationship between cell dimension, proportion of tissues in the phloem and age was observed; the effect of stem height position was statistically significant for sieve cell length, fiber–sclereid length and wall thickness with a decrease from the base to the top. The rhytidome thickness increased with cambial age: At the stem base (45–50 years of cambial age), the bark includes a rhytidome of about 3 cm thickness corresponding to 84% of the bark, with 5–8 periderms, containing nearly 50% of cork. The cork cells were thin-walled and oriented in radial rows, and the occurrence of thick-walled lignified cells was associated with the increments of the phellem layer. In the youngest periderm, the occurrence of phellem cells with empty lumens and thin suberized walls started at 25–30 years of cambial age. The results show that trees with 45–50 years of age and their logs up to 5 m of height may be suitable for bark and cork exploitation.

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Introduction

Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) is native to North America, where it is planted and exploited as a timber species. Today, it is also an important economic species in Europe due to its fast growth rate, high reproduction capacity and adaptation, low number of pests and diseases, and good-quality wood (Da Ronch et al. 2016). The bark characteristics contribute to the species success and adaptation by its enhanced protective and defense functions, for example, shielding from water loss, adverse external temperatures and fire, as well as from pathogen entry and mechanical damage (Morris and Jansen 2016).

Tree barks as residue from forestry and primary wood processing are a valuable raw material for biorefineries due to their structural and chemical diversity (Le Normand et al. 2014; Pereira and Knapic 2017). Recent research has looked into the characterization of barks in view of their valorization as a material and for chemical compounds with high added value, which can be used further in chemical source or pharmaceutical industry (e.g., Miranda et al. 2012, 2013a; Baptista et al. 2013; Mota et al. 2016).

One of the striking features of Douglas-fir bark is its substantial proportion of cork in the rhytidome (Ferreira et al. 2015, 2016; Cardoso et al. 2017). Cork is a cellular material with a unique set of properties that allows its use as sealant, insulator, damper and protective surfacing, for example, as the world-known wine corks that are at the basis of its economic importance as raw material (Pereira 2015). The main source of cork is *Quercus suber* (Pereira 2007), but other species also contain appreciable proportions of cork (Leite and Pereira 2017), for example, *Quercus variabilis* (Miranda et al. 2013b), *Quercus cerris* (Şen et al. 2010, 2011), *Plathymenia reticulata* (Mota et al. 2016), *Betula pendula* (Pinto et al. 2009; Ferreira et al. 2017).

Potential use of Douglas-fir bark as a raw material has already been analyzed and presented in earlier papers published in the 1950s of last century (Kurth and Kiefer 1950; Hergert and Kurth 1952; Grillos and Smith 1959; Hall 1971; Krahmer and Wellons 1973; Ross and Corden 1974; Litvay 1976; Litvay and Krahmer 1977; Dougal 1981). The first description of Douglas-fir bark structure based on light microscope observations was presented in 1954 (Chang 1954), and detailed illustrations using transmission electron micrographs were reported in 1981 (Dougal 1981).

Although bark formation is a known process involving the activity of the vascular cambium and the phellogen (Angyalossy et al. 2016), several aspects of bark development have been scarcely studied, namely the timing of the formation of different bark tissues, especially of periderms, or the age-related variation in structural features, i.e., secondary changes in older bark tissue and the rhytidome accumulation.

For Douglas-fir, the origin and development of the bark tissues in different stages of development were studied (Grillos 1956) as well as the variation in the bark structure and development with tree growth and environmental factors (Ross and Krahmer 1971).

Age-related structural changes in bark have been reported for some other species, for example, *Quercus robur*, *Ulmus glabra*, *Populus tremula* and *Betula*

pendula (Trockenbrodt 1994), *Eucalyptus globulus* (Quilhó et al. 1999, 2000), and *Quercus faginea* (Quilhó et al. 2013). In recent years, the development and secondary changes in bark tissues of *Quercus petraea* were also studied by Gricar et al. (2015). In general, the structural changes in the bark are related to age and give rise to a typical within-tree pattern of axial variation that is characterized by an increase in sclerification and cell dilatation from top to bottom of the tree, as well as by rhytidome accumulation (Pereira et al. 2010).

This paper reports the results of a detailed analysis of the bark structure and development within the stem of Douglas-fir trees growing in two sites in Portugal in relation to cambial age, concerning the phloem and rhytidome formation. Specific goals of this study were to investigate the age-related trends in the bark structure of Douglas-fir and evaluate the contribution of individual tissues; to estimate the age at which rhytidome was first observed and the formation rate of the successive periderms, and to evaluate the relationship between cork proportion and cambial age. These results will be relevant for a potential industrial exploitation of Douglas-fir bark with a valorization of its cork component, including designing management orientations at forest and primary conversion levels.

Materials and methods

The study was performed on the bark of 20 Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) trees that were randomly selected in two state-owned stands in northern and central Portugal at the time of harvest for the timber industry. The stands resulted from the afforestation programs carried out by the state in the middle of last century targeting forest densities around 1000-1200 trees ha^{-1}.

One stand (here named Cabreira) is located in the Forest Perimeter of Serra da Cabreira, Cabeceiras de Basto (40°21′28.5″N, 07°27′07.2″W, 850 m of altitude), where the annual rainfall ranges from 1600 to 2000 mm, with 50% of precipitation during the winter season, and a mean annual temperature from 7.5 to 10 °C; it is a mixed stand, with Douglas-fir as the dominant species but including also *Pinus pinaster*, *Pinus sylvestris*, *Cupressus lusitanica*, *Chamaecyparis* spp., and *Betula pendula*. The other stand (here named Estrela) is a pure stand, located in the Forest Perimeter of Sarzedo, in the region of Serra da Estrela, Covilhã (41°35′18.0″N, 8°01′00.6″W, 930 m of altitude), where the annual rainfall ranges from 1200 to 1400 mm, with 42% of precipitation during the winter, and a mean annual temperature from 7.5 to 10 °C.

The selected trees (ten trees per site) had an average diameter at breast height of 61 cm in both sites and a total height of 29 m and 35 m at Cabreira and Estrela, respectively. The trees were felled after the end of the growth season (September–January) and bucked into 2.5-m-long logs following the sawmill's requirements. Tree age (as given by ring counting at stem base) ranged from 43 to 50 years and 39 to 64 years at Cabreira and Estrela, respectively.

Cross-sectional disks with approximately 10 cm thickness were taken from each tree along the stem at six height levels at intervals of approximately 5 m of stem height that corresponded to a difference of tree age between height levels of ca.

6 years. The sampling was made carefully in order to maintain the total bark layer in each stem disk. The stem disks were air-dried indoor under well-ventilated conditions. The surface was smoothed by sanding prior to observations and measurements. Ring counting was made along two opposing radii on each observed cross section.

Images of the cross-sectional disks were acquired with an image analysis system that included a digital seven mega pixels in macro-stand solution set on an acquisition Kaiser RS1 Board with a controlled illumination apparatus, connected to a computer using AnalySIS® image processing software (AnalySIS Soft Imaging System GmbH, Münster, Germany, version 3.2). The determination of the radial thickness of phloem, periderm or rhytidome in the bark was made on two opposite radii as well as the proportion of cork and phloem delimitating in the rhytidome the areas of each tissue. Counting of the number of periderms in the rhytidome and measurement of the thickness of the phloem and cork layers in the rhytidome were made in three randomly selected directions on the same images with the software Leica Qwin Plus.

Analysis of bark anatomical features was made at the six stem height levels of each tree from Cabreira; small bark specimens were cut, impregnated with DP 1500 polyethylene glycol, and transverse and longitudinal microscopic sections of approximately 17 µm thickness were prepared with a Leica SM 2400 microtome using Tesafilm 106/4106 adhesive for sample retrieval (Barbosa et al. 2010). The sections were stained with a double staining of chrysoidine and astra blue. Sudan 4 was used for selective staining of suberin. The sections were mounted on Kaiser glycerine, and after 24 h drying, they were submerged in xylol during 30 min to remove the Tesafilm, dehydrated with 96% and 100% ethanol, and mounted on Eukitt. Individual specimens of bark samples were also macerated in a mixture (1:1) of 30% hydrogen peroxide and acetic acid in a 60 °C oven for 48 h and stained with astra blue.

The parameters measured and the number of measurements for each specimen at each tree height level were as follows: length, width and cell wall thickness of 40 fiber–sclereids, measured in the macerated material; length and tangential diameter of 30 sieve cells measured in the macerated material and in the transverse section, respectively; and height (in µm and number of cells) of 30 rays determined in the tangential section. All measurements were taken using a microscope and a semiautomatic image analyzer system (Leica Application Suite).

The proportion of tissue types was calculated in the transverse section on five randomly selected areas from cambium to periderm using the image analysis system coupled to a microscope; a grid with 48 points was placed over each image, and tissue types (sieve cells, axial parenchyma, fibro-sclereids, sclereids and rays) were counted and converted into a percentage of the total area (Quilhó et al. 2000). The axial parenchyma cells and the sieve cells were quantified together, since the sieve cells were only distinguished in conducting phloem by their radial alignment and axial diameter. In transverse section, the collapse of the sieve cells in non-conducting phloem did not allow their recognition.

The light microscopic observations were made using a Leica DM LA microscope, and photomicrographs were taken with a Nikon Microphot-FXA. Additionally, small cubes of bark with approximately 5 mm of edge were cut with a sharp

razor blade at each of the six stem height levels, and their surfaces were examined with a scanning electron microscope Hitachi TM 3030 Plus at 5 kV with different magnifications, and the images of cubical crystals and starch grains were recorded in digital format.

Significant differences in stand characteristics were assessed with paired *T* tests. Analyses of variance (ANOVA) were performed for bark anatomical features to assess the effects of site, trees, stem height levels and their interactions. Statistical calculations were carried out with IMB SPSS Statistics v19 software.

The terminology follows IAWA list of microscopic bark features (Angyalossy et al. 2016).

Results

Bark structure

The external appearance of Douglas-fir bark varied within the tree along the stem: It was thick, rough with deep fissures and reddish brown in color in the older portions at the stem base and lower levels and rather smooth and grayish at the top of the tree (Fig. 1).

Figure 2 illustrates the layers of phloem, periderm and rhytidome that comprised the Douglas-fir bark and could be macroscopically observed in cross sections. In the lower part of the stem, the rhytidome was substantial with numerous and sinuous periderms (Fig. 2a–c), varying from thin lines to large bands, that isolated patches of phloem tissue visible to the naked eye. The light cream-colored periderm (Pr) tissues differed clearly from the brown phloem tissue (Phm) in the rhytidome (Rt).

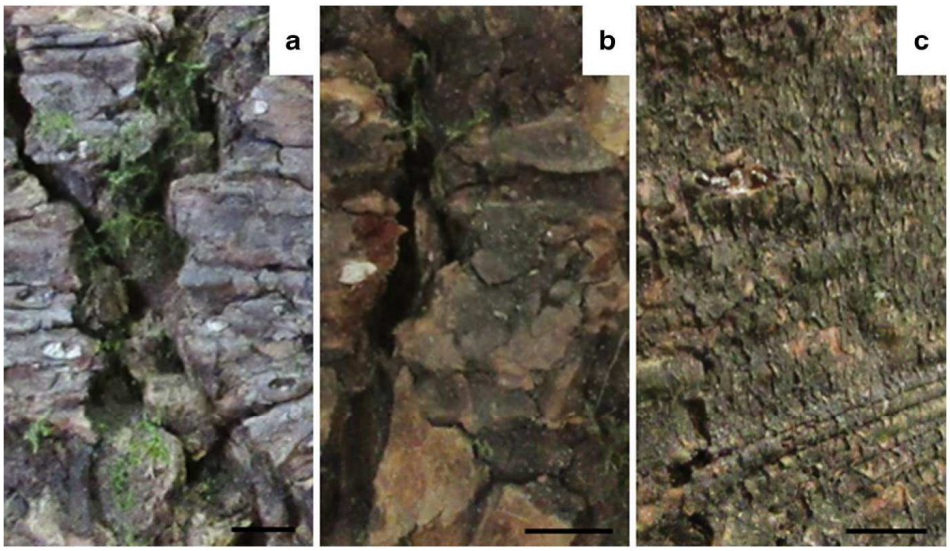

Fig. 1 Images of Douglas-fir bark showing the macroscopic external appearance at different stem height levels and cambial ages. **a** Base (45 years); **b** 6 m (36 years); and **c** 19 m (17 years) (bar: 1 cm)

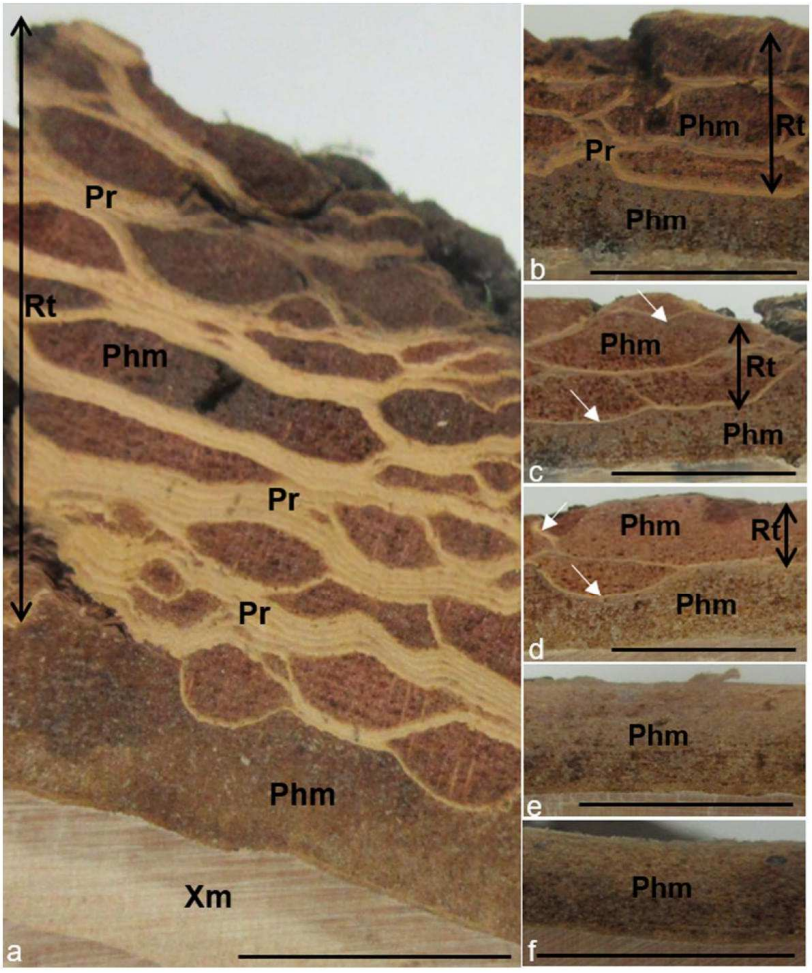

Fig. 2 Transverse section of Douglas-fir bark at different height levels and cambial ages. **a** Stem base (45 years); **b** 6 m (36 years); **c** 11 m (29 years); **d** 13 m (25 years); **e** 19 m (17 years); and **f** 21 m (12 years). Rt—rhytidome; Pr—periderm; Phm—phloem; Xm—xylem; and white arrow—periderm (bar: 1 cm)

In contrast, at the top of the tree, only one single and more or less continuous periderm was present (Fig. 2e, f). With age, at the stem base and lower stem levels, the tree develops in the phloem fairly thick cork layers and many short fiber–sclereids (Fig. 2), where the bark has flaky patches of cork bound together by sclerenchyma fractured in furrows, while it is rather smooth and only slightly fissured in the younger bark at the stem top levels.

The bark structure and radial dimensions at the different stem height levels were similar in all the studied Douglas-fir trees (Table 1) with small significant differences ($p < 0.05$) between sites, height levels and trees. The largest bark thickness was at the stem base (on average 32.7 mm), decreasing to the top (on average 5.5 mm). The proportion of the rhytidome in terms of its radial thickness proportion

Table 1 Variation in the radial thickness of total bark, phloem, and rhytidome and proportion of rhytidome along the stem of Douglas-fir trees from two sites

	Height (m)					
	Base	5	10	14	19	24
Cabreira						
Total bark (mm)	34.5 ± 9.9	17.6 ± 3.6	14.1 ± 2.4	9.7 ± 3.2	5.9 ± 1.5	3.8 ± 0.7
Phloem (mm)	5.9 ± 1.2	4.2 ± 1.4	4.6 ± 1.6	5.1 ± 1.8	5.6 ± 1.2	3.8 ± 0.7
Rhytidome (mm)	28.6 ± 9.3	13.4 ± 2.8	9.4 ± 3.1	4.6 ± 4.3	0.2 ± 1.3	0.0 ± 0.0
Rhytidome (%)	82.0 ± 4.8	76.1 ± 5.2	65.2 ± 18.4	37.9 ± 34.7	2.2 ± 12.1	0.0 ± 0.0
Estrela						
Total bark (mm)	30.8 ± 9.7	17.3 ± 5.7	14.3 ± 2.3	12.2 ± 1.9	8.8 ± 4.1	6.4 ± 4.2
Phloem (mm)	4.2 ± 1.2	3.3 ± 1.6	3.2 ± 0.9	3.5 ± 1.0	4.9 ± 1.7	4.3 ± 0.6
Rhytidome (mm)	26.6 ± 9.1	14.0 ± 4.5	11.1 ± 2.4	8.7 ± 1.7	3.9 ± 5.0	2.2 ± 4.4
Rhytidome (%)	85.9 ± 4.1	80.9 ± 4.2	77.5 ± 4.6	71.3 ± 10.8	30.0 ± 37.3	13.5 ± 28.5

Mean of ten trees and standard deviation

in relation to the total bark thickness was highest at the stem base and lowest at the top (on average 84% vs. 9%). The rhytidome thickness increased with the cambial age (Fig. 3) from approximately 5 and 4 mm at 25 and 27 years to 29 and 27 mm at

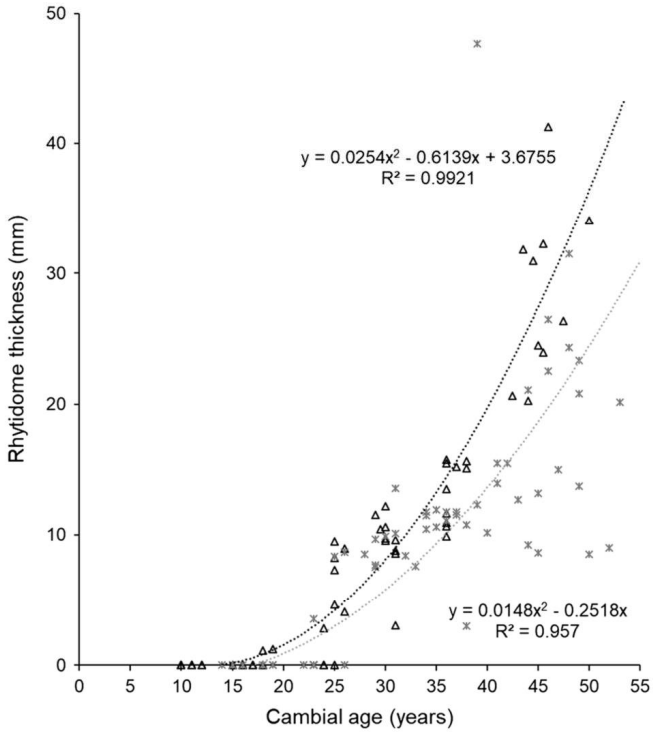

Fig. 3 Variation with cambial age of the radial thickness of rhytidome in the bark of Douglas-fir trees from two sites. Triangles and black dashed line—Cabreira; stars and gray dashed line—Estrela

45 and 50 years, respectively, for Cabreira and Estrela (Fig. 3). The phloem thickness increased until 18 and 25 years for Cabreira and Estrela, respectively, and declined then until approximately 35 years and increased subsequently.

The number of periderms contained in the rhytidome was highest in the lower part of the stem (five and eight in Cabreira and Estrela, respectively) and decreased to the upper part of the trees (Fig. 4a). The number of periderms increased with cambial age, in particular after 30 and 35 years at Cabreira and Estrela, respectively (Fig. 4b). In Cabreira, the first periderm that was formed in the phloem tissue appeared at a cambial age of 25 years and died between 30 and 37 years; in Estrela, the first periderm was formed and replaced earlier, because at a cambial age of about 21 years, the trees had already one periderm (Table 2).

The proportion of cork in the bark of Douglas-fir trees was in relation to the development of the rhytidome; i.e., it increased with cambial age and decreased

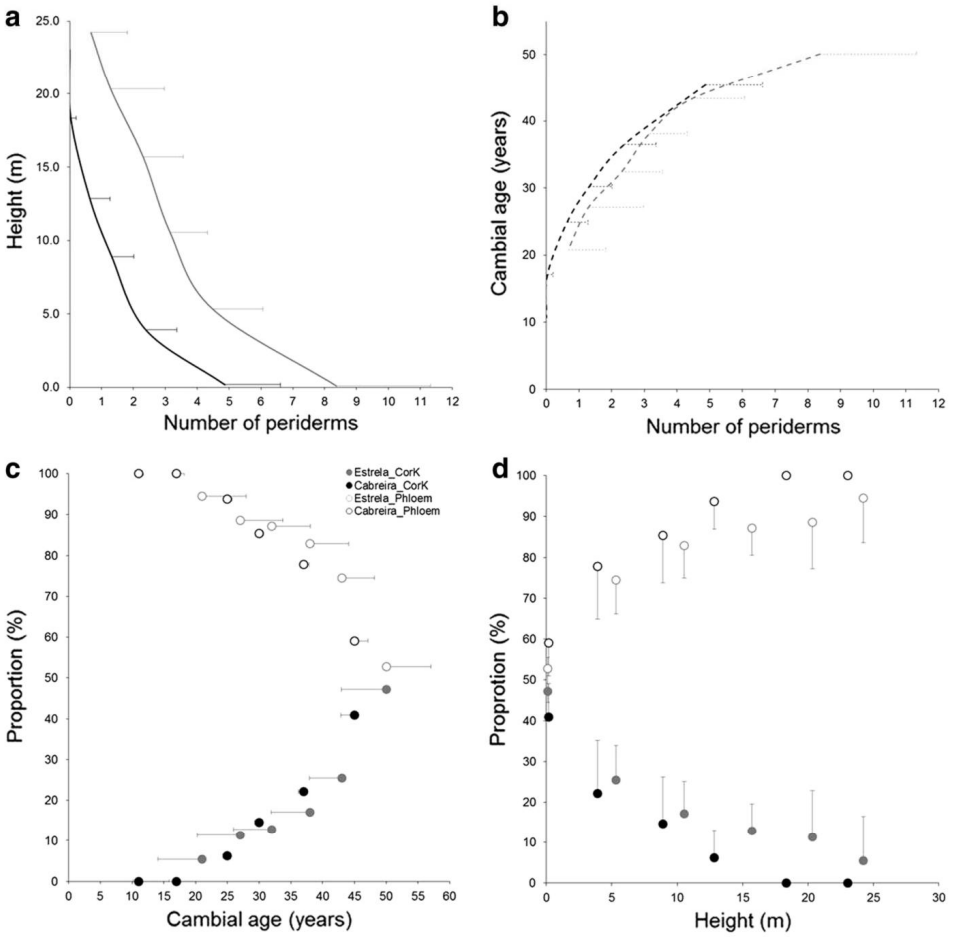

Fig. 4 Variation in the number of periderms (**a**, **b**) and proportion of cork and phloem (**c**, **d**) with stem height level and cambial age in rhytidome of Douglas-fir trees from two sites. Mean of ten trees and half standard deviation as bar. (**a**, **b**: black lines—Cabreira; gray lines—Estrela)

Table 2 Variation in the number of periderms along the stem of Douglas-fir trees from two sites

Height (m)	Cabreira		Estrela	
	Tree age (years)	Number of periderms	Tree age (years)	Number of periderms
Base	45	5 ± 2	50	8 ± 3
5	37	2 ± 1	43	4 ± 2
10	30	1 ± 1	38	3 ± 1
14	25	1 ± 1	32	2 ± 1
19	17	0 ± 0	27	1 ± 2
24	11	0 ± 0	21	1 ± 1

Mean of ten trees and standard deviation

along the stem height. No significant difference ($p > 0.05$) was found between sites (Fig. 4c, d): Cork represented nearly 50% of the rhytidome at a cambial age of 50 years and 15% at 30 years. The proportion of phloem varied correspondingly with an opposite trend (Fig. 4c, d).

The bark structure was similar in all the trees sampled from both sites; the site did not influence the proportion of cork but affected the thickness of total bark, phloem and rhytidome, and the rhytidome proportion.

Bark anatomical features

The Douglas-fir bark anatomical structure was analyzed by light and scanning electron microscopy. The bark includes a secondary phloem comprising the conducting

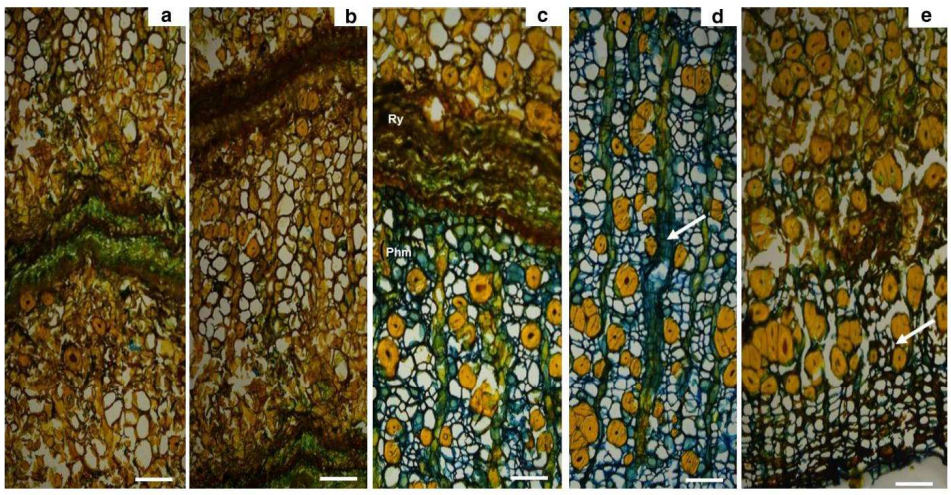

Fig. 5 Transverse section of Douglas-fir bark at the base of the tree. **a**, **b** Rhytidome; **c** non-conducting phloem (Phm) and rhytidome (Ry); **d** non-conducting phloem (middle), dilated ray (arrow), and **e** fiber–sclereids appeared about ten sieve cell layers above cambium (arrow) (bar: 50 µm)

and non-conducting phloem, and a rhytidome that includes the inner and sequent periderms interspersed with layers of phloem tissue (Fig. 5).

The conducting phloem, located near the vascular cambium, was composed of living sieve cells with turgid Strasburger cells, axial and radial parenchyma; no typical phloem fibers were found, and instead, short and very thick-walled cells with minute lumen, like fiber–sclereids, were present (Fig. 6). The transition of conducting to non-conducting phloem started early, i.e., close to the cambial region, and was marked by the collapse of sieve cells; the parenchyma cells and sclerenchymatic tissue (fiber–sclereids and sclereids) became the prominent tissue in the non-conducting phloem. Annual increments could be noticed in the phloem by tangentially compressed cells (Fig. 6a).

The sieve cells were arranged in regular rows of about 3–10 cells and interspersed by parenchyma cells and fiber–sclereids (Fig. 6a). The sieve cells were elongated with unlignified thin walls and sieve areas on the radial walls; the sieve areas were mostly oval to elliptical, in single rows or sometimes arranged in pairs, including numerous and distinct pores (Fig. 6b).

The axial parenchyma cells were thin-walled, approximately circular and somewhat rectangular near the cambium, mostly arranged in 2–3 discontinuous tangential lines; they were sometimes difficult to distinguish from sieve cells due to the similar size and outline (Fig. 6a). Abundant cubical crystals and ergastic contents filled the cell lumen, as well as starch grains (Fig. 7a).

The rays were of two types: uniseriate and homocellular, and fusiform rays containing resin ducts with distinct border formed by thin-walled epithelial cells (Fig. 6c). Strasburger cells appeared in the margins of the rays in the conducting phloem. Ray cells contained dark brown substances.

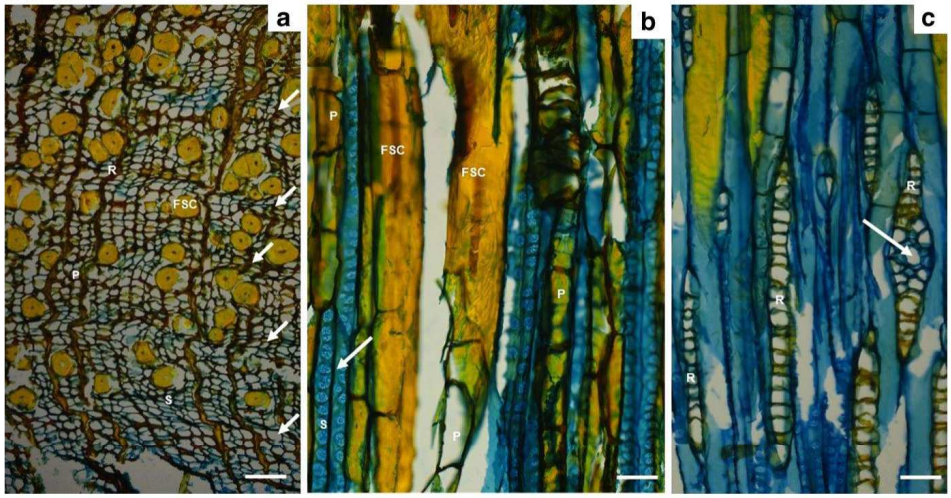

Fig. 6 Anatomical features of Douglas-fir bark in transverse (**a**), radial (**b**), and tangential (**c**) sections. **a** Sieve cells (S), increment growth (arrows), axial parenchyma cells (P), rays (R), fiber–sclereids (FSC). Annual increments were noticeable in the phloem (arrows). **b** Sieve cells (S) with sieve areas (arrow), axial parenchyma (P) and fiber–sclereids (FSC); **c** Uniseriate rays (R) and fusiform ray with a transverse resin canal (arrow) (bar: **a** 125 µm and **b**, **c** 50 µm)

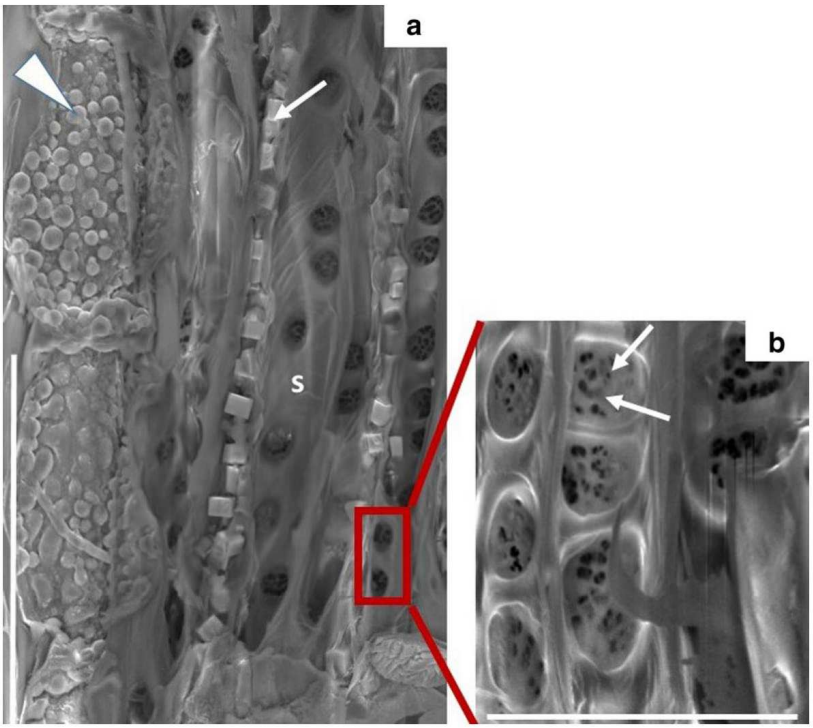

Fig. 7 Anatomical details in the phloem of the bark of Douglas-fir observed under SEM. **a** Abundant cubical crystals (arrow) and starch grains (arrowhead); sieve cells (S); **b** sieve areas with numerous pores (arrows) (bar: **a** 100 µm and **b** 30 µm)

On transverse sections, the fiber–sclereids were mostly solitary and diffusely distributed (Figs. 6, 7), with occasional groups of 2–3 cells; they appeared early, i.e., about 6–8 cells in the first-formed phloem tissue (Figs. 5e, 6a), more or less oval in cross section and becoming more irregular toward the periderm, with very thick and polylamellated walls and a very narrow lumen. Figure 6b shows the fiber–sclereids in tangential view. After maceration (Fig. 8), these cells appeared elongated with pointed ends, and some were branched and forked; branched sclereids (brachysclereids) occurred in the external portion of phloem, mainly in the cortex.

Two different forms of sclerenchyma were identified in the present work: fiber–sclereids and sclereids (Fig. 8). The latter, mostly brachysclereids, were short and ramified (Fig. 8b) and developed mainly in the non-conducting phloem, cortex and periderm. In the present work and given the lack of ontogenetic studies for the precise classification of cell type, the term fiber–sclereids was preferably used for the cells with an elongated form and thick wall (Fig. 8a). The first sclerenchyma cells appeared early in the bark of Douglas-fir, and thick cells were found after 6–8 cells in the first-formed phloem tissue (Figs. 5e, 6a).

The periderm (Pr) is made up of phellem or cork (Phlm), phellogen and phelloderm (Fig. 9). The phellem layer comprised a variable number of mainly thin-walled and somewhat rectangular cells in radially oriented rows (early cork cells), although thick-walled lignified cells also occurred (late cork cells) and were associated with

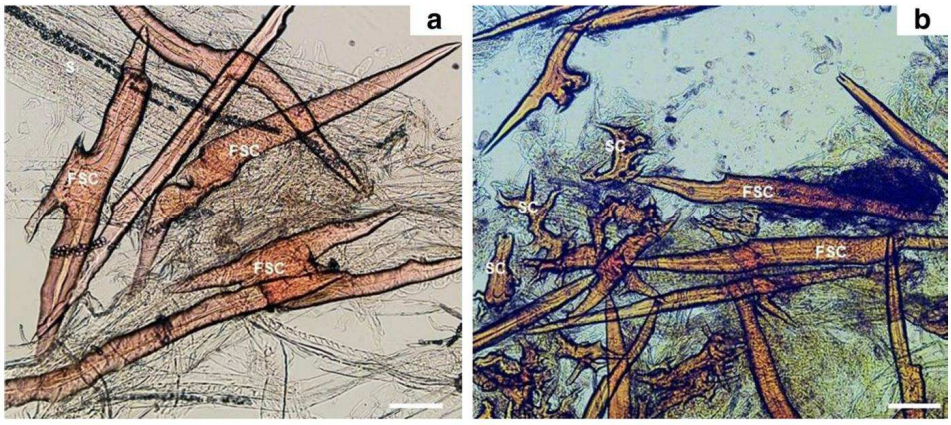

Fig. 8 Dissociated phloem cells of Douglas-fir bark. Sieve cells (S), fiber–sclereids (FSC) and brachy-sclereids (SC) from the base (**a**) and from the top (**b**) of the tree (Bar: 125 µm)

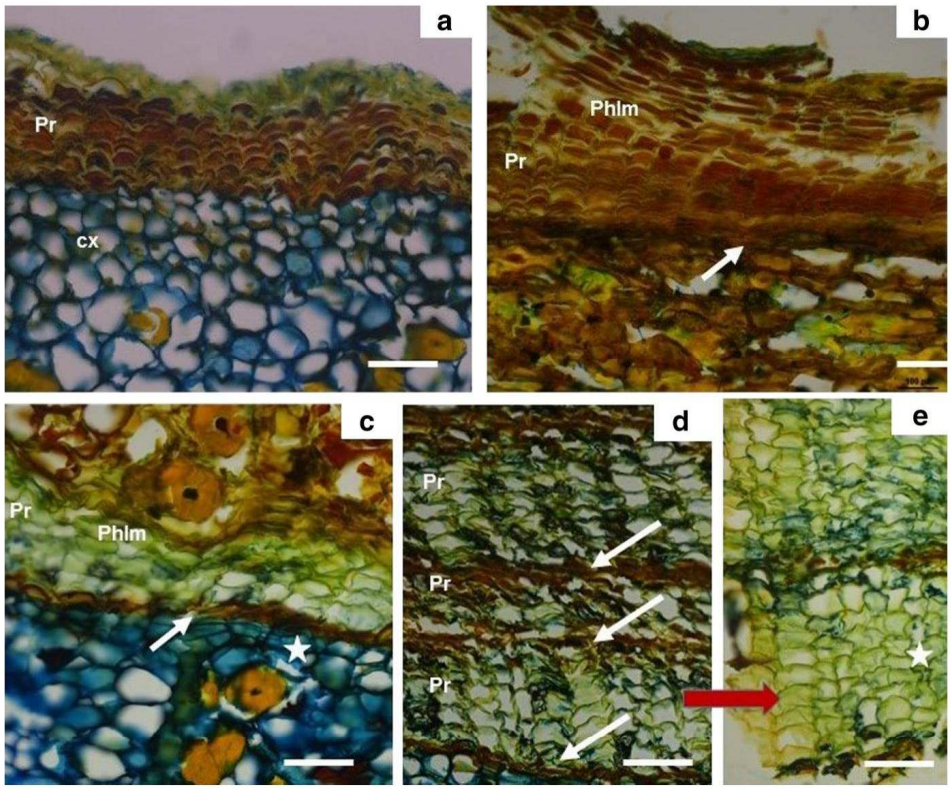

Fig. 9 Different stages of periderm development in Douglas-fir bark in transverse section. **a** An initial periderm (Pr) and beneath the cortical cells (cx) observed in the top of the tree. **b**, **c** Degrees of periderm (Pr) development including a layer of phellem cell (phlm); differentiation of phelloderm cell (1–2 cells) (star) by the phellogen (arrow) in the base of the tree. **d** Annual increments in the phellem (arrows). **e** Phellem or cork cells (star) (bar: 50 µm)

the annual phellem increments (Fig. 9d, e); small crystals were observed inside phellem cells. The phelloderm comprised radially aligned thin- to thick-walled cells. The rhytidome included a variable number of periderms according to the cambial age (Fig. 2), forming layers with outward curving edges that merged with older periderms; the phloem layers were thus isolated in a scale-type pattern, and the outer bark formed a scale bark.

The cork tissue comprised extensive areas of crushed phellem cells that formed a very compressed and compact structure alternated by patches of uncompressed cells with the typical arrangement of cork cells. As a result of the different intensities of compression, the cork cell walls showed different degrees of folding, from heavy buckling to only little undulation (Fig. 9d). These aspects could be recognized along the different heights of the stem, although they were more visible at the bottom where the proportion of phellem was higher. Late cork cells with lignified thick walls were observed that were associated with annual increments in the phellem (Fig. 9d, e).

Bark structure varied within the tree with cambial age at the various height levels, mostly regarding rhytidome and periderm development (Figs. 2, 9). Qualitative changes in the non-conducting phloem were also observed, mostly regarding tissue disarray (Fig. 4), mainly due to the enlargement of the axial parenchyma cells. The cortex (primary phloem) was maintained until the replacement of the first periderm (Fig. 9) at 30–37 years of cambial age.

The rhytidome thickness and its number of periderms increase with age, i.e., from top to stem base (Figs. 3, 4), and consequently, the amount of cork is higher in the bottom part of the tree: the cork represented nearly 50% of the rhytidome at a cambial age of 50 years (Fig. 4c, d). The occurrence of phellem cells with empty lumens and thin suberized walls started at approximately 25–30 years of cambial age, but a substantial proportion of cork was found at older ages of about 50 years.

Age variation in bark anatomy

The bark structural features showed changes that increased gradually from the upper stem levels of the trees downwards with most structural differences occurring at the base and lower levels of the tree stem, i.e., at older ages (Fig. 4c, d). At these levels, corresponding to cambial ages of over 50 years, there was a pronounced disarray of the phloem tissues from the beginning of the non-conducting phloem outwards, mainly due to the enlargement of the axial parenchyma cells and the increase in number and diameter of sclerenchyma cells. The radial alignment of the sieve cells that was evident near the cambium was subsequently lost toward the outside by distortion through cell collapse (Fig. 5c, d). In the non-conducting phloem and toward the periderm, the axial parenchyma cells expanded and tended to lose the initial alignment (Fig. 5c). In contrast, the rays that extended linearly through the conducting phloem became gradually distorted and slightly dilated (Fig. 5d). In the young levels near the top, a cortex was observed beneath the inner periderm (Fig. 9a); the cortex was preserved until approximately 26 years of cambial age.

The periderms showed age-related differences, namely regarding their number, which increased with age (Fig. 4a, b), the number of cells in each phellem layer, their outline and content. At the top, at cambial age of 10 years, only one periderm was present with a few phellem cells (5–6 cells in a row), rounded and filled with heavily stained materials (Fig. 9a). The occurrence of phellem cells with empty lumens and thin suberized walls, as represented in Fig. 9b–e, started at approximately 25–30 years of cambial age; at the tree base, corresponding to cambial ages of over 40 years, a broad phellem layer (up to 10 cells) could be observed. After approximately 30 years, annual increments could be noticed in the phellem layer: the initial cells of the phellem were large, thin-walled and free of contents, followed by a few crushed cells, occasionally thick-walled and with heavy deposits (Fig. 9d). Each phellogen mother cell produced usually two or three phelloderm cells often containing dark substances.

Cell biometry and tissue proportion

Cell sizes and amount of tissues varied within and between trees, as shown in Table 3.

Sieve cells were on average 20 µm in diameter and 2293 µm in length (Table 3); only cell length showed significant differences between trees ($p < 0.05$). Younger barks had in general thinner and shorter sieve cells, although the within-tree variation did not show a regular gradient. The cell length slightly increased from stem base to approximately 5 m, followed by a decreasing trend to the upper part of the stem. The effect of height position was statistically significant ($p < 0.05$) for sieve cell length. The sieve cells were about two times longer than fiber–sclereids (Table 3).

Table 3 Cell biometric data and proportion of tissues in the phloem in the bark of Douglas–fir regarding tree average (mean, standard deviation, minimum and maximum) at the stem base and top levels

	Tree average		Base	Top
	Mean (standard deviation)	Min–Max		
Sieve cells				
Tangential diameter (µm)	19.6 (6.4)	6.6–44.6	20.0	19.1
Length (µm)	2292.9 (677.5)	351.2–4575.1	2330.3	1943.0
Proportion (%)*	68.0 (6.3)	61.7–76.2	58.9	74.3
Fiber–sclereids				
Length (µm)	1277.4 (236.2)	449.3–3582.9	1195.6	1174.1
Width (µm)	76.7 (13.7)	38.0–120.7	78.6	76.1
Wall thickness (µm)	31.6 (6.5)	13.2–56.8	13.7	12.7
Proportion (%)	22.5 (5.8)	14.8–29.0	25.8	19.6
Rays				
Height (µm)	184.4 (92.8)	41.1–947.0	219.9	178.5
Proportion (%)	10.3 (1.5)	8.8–12.5	15.7	6.3

*Proportion of sieve cells and axial parenchyma

The diameter of sieve cells did not vary significantly along the tree, contrary to what occurred for length with the longest sieve cells found at the stem base (Table 3).

Fiber–sclereids were on average 77 µm in diameter, 1277 µm in length and 32 µm in wall thickness (Table 3) with significant between-tree differences ($p < 0.05$) of length and wall thickness. The axial variation in fiber–sclereid width and wall thickness showed a decreasing pattern from the base to the top with some fluctuations, and length increased initially and decreased at the top. The fiber–sclereid length and wall thickness varied significantly within the tree ($p < 0.05$) but with little dimensional variation. Differentiation of parenchyma cells into fibro-sclereids included elongation and wall thickening and additional deposited cell wall layers, thereby developing a multilayered cell wall (Figs. 6, 9).

Height of phloem rays averaged 184 µm (Table 3), with significant differences between trees and height levels ($p < 0.05$), and was mostly uniseriate and eight cells high on average. Ray height showed a decreasing trend from the base to the top of the trees.

The proportion of the different tissues in the phloem was determined at each height level (Table 3). The axial parenchyma together with sieve cells represented the major tissue followed by the sclerenchyma tissue (fiber–sclereids and sclereids) ranging between 62–76 and 15–29%, respectively; rays corresponded to 9–13% of the phloem. The different types of cells varied within the tree but not always in the same manner and extent. The axial parenchyma and sieve cells were more abundant in young barks, and their proportion increased toward the top; in contrast, fiber–sclereids and sclereids showed the highest proportion at stem base corresponding to higher cambial age. The proportion of the radial parenchyma tended to decrease toward the top. The proportion of the different cell types in the transverse section (Table 3) shows a very high value of axial parenchyma and sieve cells (68%) compared to, for example, rays (10%) or fiber–sclereids (23%). The proportion of fiber–sclereids was higher toward the stem base.

Discussion

Bark structure and anatomical features

The bark of Douglas-fir is characterized by the formation of a rhytidome, and the periderms include substantial layers of cork cells (Fig. 2). These striking features of Douglas-fir bark were reported in earlier studies (Kurth and Kiefer 1950; Hergert and Kurth 1952; Ross and Krahmer 1971) and more recently regarding the characterization of the cork layers (Ferreira et al. 2015, 2016; Cardoso et al. 2017).

The observed variation in the bark external appearance along the stem of Douglas-fir (Fig. 1) is related to the specific structural features and their development with tree age (Junikka 1994). The occurrence of flaky patches of cork held together by sclerenchyma fractured in furrows in the bark of mature trees and of a rather smooth and only slightly fissured bark at the stem top levels has already been reported in the literature (Chang 1954; Ross and Krahmer 1971).

The observed Douglas-fir bark anatomical structure is generally in accordance with the first descriptions made in the literature, although a different terminology was then used to name the sclerenchyma cells, for example, fibers (Chang 1954; Patel 1975), sclereids-like phloem fibers (Einspahr et al. 1978), sclereids phloem fibers (Parameswaran 1980), or sclereids (Grillos 1956; den Outer 1967; Ross and Corden 1973; Ross and Krahmer 1971). The presence of sclereids, mostly brachysclereids, results from the modification of parenchyma cells (Angyalossy et al. 2016) and gives mechanical support to the phloem tissue (den Outer 1967). Sclerenchyma usually includes fibers, fiber–sclereids, and sclereids (Angyalossy et al. 2016), which have a different origin: Fibers arise from cambial cells, while fiber–sclereids and sclereids are formed by a secondary sclerosis of parenchyma cells (Evert 2006). The early appearance of the first sclerenchyma cells observed is in accordance with previous reports of an early formation at 15 cells away from cambium (Chang 1954), or 1–30 cells away from the cambium (Grillos 1956).

Cell biometry and tissue proportion

Sieve cells, fiber–sclereids and ray dimensions, and their proportion in the phloem varied within the tree (Table 3). This is a combined age effect on the structure of cambial cells and their derivatives, i.e., phloem mother cells and their products (Evert 2006) with influence of exogenous and endogenous regulators (Fromm 2013), and of phloem adjustment to tree radial growth. The cell dimensions (Table 3) fitted in general with the scarce available information on Douglas-fir bark.

Sieve cell length was in the range of values reported, for example, 2.5–3.7 mm (Chang 1954), 3–4 mm (Einspahr et al. 1978), and 3.0 mm (Patel 1975), and tangential diameter similar or smaller to, for example, 20 µm (Patel 1975) and 50 µm (Chang 1954). Sieve cells four times longer than sclerenchymatic cells have previously been reported (Ross and Krahmer 1971). The longest sieve cells were found at the stem base, but the variation in sieve cell diameter along the tree was small, as already observed in many other species (Iqbal and Ghouse 1983; Trockenbrodt 1994), and may reflect an increasing length of cambial fusiform initials with increasing age (Larson 1994). However, there are contradictory reports on the axial variation, for example, an increase in cell length to the top of mature Douglas-fir trees (Ross and Krahmer 1971) as well as irregularly axial variations, probably explained by the combined effect of cambial initial length, extent of anticlinal divisions and apical intrusive growth (Ross and Krahmer 1971; Ghouse and Iqbal 1977; Trockenbrodt 1994; Quilhó et al. 2000).

The dimensions of fiber–sclereids (Table 3) were in accordance with the reported range of values, for example, for diameter 50–100 µm (Einspahr et al. 1978), 50–92 µm (Grillos 1956), 50 µm (Chang 1954; Dougal 1981), 54–56 µm (Ross and Krahmer 1971), and 44–95 µm (Patel 1975) and for length 1 mm (Dougal 1981), 0.5–2.0 mm (Ross and Krahmer 1971), 0.6–1.5 mm (Chang 1954), 1–1.5 mm (Einspahr et al. 1978), 0.6–4.0 mm (Grillos 1956), and 0.65–1.65 mm (Patel 1975). The small variation in fiber–sclereids dimension within the tree was also reported in other studies (Ross and Krahmer 1971).

Ray dimensions (Table 3) were also in accordance with reported values, for example, 4–30 cells high (Quilhó et al. 2013) and 8–15 cells and 200–250 µm high (Grillos and Smith 1959). The decreasing pattern of variation in ray height toward the top of the tree is in relation to the progressive increase in size of cambial initials with age (Ridoutt and Sands 1993; Trockenbrodt 1994).

The major proportion of axial parenchyma and sieve cells compared to rays or fiber–sclereids (68% vs. 10–23%, Table 3) is according to the literature reporting that the secondary phloem in *Pinaceae* consists of up to 90% sieve cells (den Outer 1967). The higher proportion of fiber–sclereids at the stem base agrees with the intense lignification and sclerification of the cell walls that constitute the main structural features related to tree age (Trockenbrodt 1994; Quilhó et al. 1999). Rays also increased downwards like in other species (Quilhó et al. 2000).

Age-related changes

Bark structure varied within the tree with cambial age at the various height levels. The differences concerned mostly the rhytidome and periderm development (Figs. 2, 3, 4), tissue morphology, and disarray in the non-conducting phloem (Fig. 5). At the base of the trees, at older ages, where the bark thickness was largest, the rhytidome was substantial with numerous periderms separated by patches of phloem tissue (Figs. 2, 3). In contrast, at the younger ages, at the top of the trees, the bark thickness was lowest and only one single periderm was present (Figs. 2, 3).

The qualitative changes in the non-conducting phloem are a consequence of a dilatation process resulting in the bark radial increase by parenchyma cell division and expansion (Angyalossy et al. 2016) to adjust bark to the tree secondary growth. These structural changes are common in conifers, for example, *Pinus pinaster* (Parameswaran 1980) and *Pinus pinea* (Nunes et al. 1999), as well as in angiosperm tree species (Quilhó et al. 1999, 2013; Şen et al. 2011). Alteration in ray cells also occurred, but tangential cell division and their enlargement were of small magnitude, and they did not develop funnel-shaped dilatation growth as in other conifers, i.e., *Phyllocladus trichomanoides* (Chang 1954).

The maintaining of cortex until the replacement of the first periderm at 30–37 years of cambial age is in accordance with earlier observations made for Douglas-fir (Gartner 1996) that recognized a first periderm immediately underneath the surface at a cambial age between 12 and 43 years and that the cork formation occurs at a relatively early age (Hergert and Kurth 1952).

In consequence of the development of the rhytidome with cambial age, as seen by the variations along the stem height, there is an increasing proportion of cork in the bark of older trees in comparison with that of younger trees (Fig. 4). These present results support the findings that indicated for Douglas-fir bark a substantial proportion of cork from 25 to almost 50% (Kurth 1953; Ross and Krahmer 1971; Krahmer and Wellons 1973; Cardoso and Pereira 2017). The present results also are in accordance with Kurth and Kiefer (1950), Hergert and Kurth (1952) and Ross and Krahmer (1971) who considered that Douglas-fir bark presents a

great variety in thickness and cork content according to site quality, tree age, and axial position on the tree.

Recently, the structure of the cork tissue in the bark of Douglas-fir was described in relation to topological arrangement, geometry, and dimensions of the cells (Cardoso et al. 2017). The present research confirms the features of the Douglas-fir rhytidome, i.e., a substantial proportion of cork, the thin cork layers, their discontinuous distribution with interspersed phloem tissues (Fig. 2), and the substantial compression of cork cells forming a compact mass of crushed cells. The cork cells with empty lumens and thin suberized walls appeared approximately at 25–30 years of cambial age (Fig. 4). Annual increments with early cork and late cork cells could be noticed (Fig. 9), as described for cork of other species, like, for example, *Q. suber* (Pereira 2007), *Q. cerris* (Şen et al. 2011), and *Q. variabilis* (Miranda et al. 2013b).

The results obtained here on the variation in bark structure within the tree and the proportion of cork in relation to cambial age show that the valorization of Douglas-fir bark by means of a targeted exploitation of cork is possible for mature trees. The butt log until 5 m of stem height is the preferable raw material, because the substantial proportion of cork is found at older ages of about 50 years.

Conclusion

The bark anatomy of Douglas-fir trees grown in Portugal was characterized in detail for the first time including an analysis of tissue structure and proportion, cell biometry, and the development of the rhytidome with cambial age along the stem. Bark structure varied within the tree with cambial age regarding rhytidome and periderm development, tissue morphology, and disarray in the non-conducting phloem.

Douglas-fir has a thick bark that develops with age a rhytidome with several periderms containing a substantial proportion of cork. The proportion of cork in the bark increases with age and becomes interesting from a yield perspective for mature trees and older cambial ages, namely over 35 years of age, with a 50% cork content in the rhytidome at 50 years. Bark valorization is therefore advantageous to be included within the Douglas-fir timber exploitation and to consider the species as a potential cork provider for the cork industry. The integration of the Douglas-fir bark valorization in the current exploitation for sawmill processing means that it will be the lower part of the stem, for example the logs up to 5 m of height that should be directed for debarking and bark processing to recover the cork component.

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AGE VARIATION OF DOUGLAS-FIR BARK CHEMICAL COMPOSITION

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The chemical composition of Douglas-fir bark was analyzed at three stem height levels of trees with different ages from two geographical locations. Cork and phloem in the bark's rhytidome were analyzed separately at stem bottom: extractives (49.8% and 17.0%, respectively), suberin (30.1% in cork) and hemicelluloses, namely arabinose content (25.3% and. 4.8% of all monomers, respectively). Suberin composition includes α,ω-alkanoic diacids (38.6%), ω-hydroxyalkanoic acids (25.6%), alkanoic acids (18.2%), alkanols (2.2%), and aromatics (8.8%). Bark's chemical composition is age-related, namely regarding suberin content: at 45, 30 and, 17 years of age, bark contained respectively 25.4%, 2.6%, and 0.9% of suberin; 24.5%, 33.9%, and 29.8% of lignin; and 29.4%, 20.6%, and 25.7% of extractives. This difference is due to the small number of periderms and low cork content in barks with 30 or less years. When aiming at a cork-targeted valorization, the lower stem parts of mature Douglas-fir trees should be considered while the high content of polar extractives at all stem heights allows an overall potential valorization.

KEYWORDS. *Pseudotsuga menziesii*; phloem; cambial age; cork; outer bark; suberin

INTRODUCTION

Under the principles of zero waste and full resource use of circular economy, tree barks are potential resources, namely as raw materials for several applications within biorefinery platforms. The structural and chemical diversity of barks allows several valorization pathways, while their readily availability as a residual stream at wood industries is an advantage for the economic feasibility.[1,2]

Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) is a valuable timber species that is exploited to a large extent in North America, where large quantities of bark residues are available at mill sites. The economic potential of Douglas-fir bark was recognized early, partly triggered by its high cork content.[3–5] Cork is a valuable industrial material with an interesting set of properties given by its specific structure and chemistry.[6] Commercial cork is known as derived from the cork oak tree (*Quercus suber*) but barks of other species may also contain high proportions of cork, as it is the case of Douglas-fir.[2]

The use of the cork component from other barks requires an appraisal of their structural and chemical features since these are the underlying rationale for the valued and specific cork properties.[7] Such studies were done for the cork of several species such as *Quercus cerris*,[1,8–10] *Quercus variabilis*,[11,12] *Betula pendula*,[13,14] and *Plathymenia reticulata*.[15] Douglas-fir cork was also investigated at anatomical and chemical levels,[16–18] showing specific typical features of cork, although with some differences in relation to the commercial *Q. suber* cork: Douglas-fir cork cells are larger, collapsed in some regions, and contain a higher content of extractives. The cork is present in

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the Douglas-fir outer bark as discontinuous layers corresponding to the successive periderms that are interspersed with phloem, thereby requiring a selective fractionation to separate both tissues if cork targeted applications are envisaged.[19,20] A similar structure is present in *Q. cerris* bark, also showing discontinuous layers of cork,[9,10] and for which it was possible to separate cork-only fractions by using trituration followed by granulometric and densiometric separation.[21] This is of special relevance when the full use of bark is the objective, thereby requiring separation of phloem and cork since they have very different cellular and chemical compositions that point out to specific target applications.[17,22,23]

Bark age is also important when considering its potential valorization since it is known that the age-related radial increase of bark is accompanied by structural, and eventually chemical, changes. Douglas-fir bark develops radially with tree age and the thickness of the outer bark, the number of periderms and therefore the amount of cork increase with cambial age, leading to bark structural differences along the stem height.[16] The highest cork proportion is found at the stem base e.g. a cork proportion of about 50% of the outer bark is found in the bottom stem logs of trees harvested for the saw milling industry while in the upper portions of the stem the bark has not yet formed an outer bark and therefore no significant cork amounts are present.[19]

Age also influences the chemical composition of bark, namely regarding content and profile of extractives.[24,25] However, little is known on the age-related chemical variation of bark structural components and, to our knowledge, no such studies were made in cork-rich barks.

The present study contributes to cover this knowledge gap by analyzing the chemical composition of Douglas-fir bark at different ages with a sampling taken at three stem height levels of mature trees at the time of harvest in two locations. Our main goal is to have chemical information that will allow designing valorization pathways for the different parts of the whole stem bark within a biorefinery.

MATERIALS AND METHODS

The bark samples were obtained from Douglas-fir (*P. menziesii* (Mirb.) Franco) trees randomly selected from two state-owned stands. One was a mixed stand located near Serra da Cabreira (northern of Portugal, 40°21′28.5″N; 07°27′07.2″W, 850 m altitude) with an annual rainfall of 1600–2000 m and a mean temperature of 7.5–10 °C; the other was a pure stand near Serra da Estrela (center of Portugal, 41°35′18.0″N; 08°01′00.6″W, 930 m altitude) with an annual rainfall of 1200–1400 m and a mean temperature of 7.5–10 °C.

The mean age of the selected trees (given by ring counting at stem base) was 45 and 50 years, respectively at Cabreira and Estrela. In both sites, the trees had an average diameter at breast height of 58 cm (62–56 cm) and a total height of 28 and 35 m at Cabreira and Estrela respectively.

A cross-sectional disc was taken at three height levels: base, middle (10 m of height) and top (20 m of height). At these height levels, the bark proportion corresponded on average to 15%, 9%, and 9% of the stem cross-section, respectively, and to cambial ages of 45–50, 30–38, and 17–27 years.

The barks were manually removed and air-dried, protected from light in a well-ventilated indoor room. A portion of each bark was ground individually in a cutting mill (Retsch SM 2000) using an output sieve of 10×10 mm, followed by one of 2×2 mm and fractionated with a vibratory system (Retsch AS 200basic) with standard sieves. After sieving, the 40–60 mesh (0.425–0.250 mm) fractions were collected for chemical analysis. Three trees were analyzed for each site.

The bark at the stem base of two trees (one from each site) was further sampled by manually separating the cork and the phloem fractions of the rhytidome. The cork and phloem layers were clearly distinguished visually by their different color and they were carefully separated using a scalpel. They were ground using a small grinder to particles

below 40 mesh and kept for chemical analysis.

Summative Chemical Characterisation

Chemical summative analyses included determination of ash, extractives soluble in dichloromethane, ethanol and water, suberin, Klason and acid soluble lignin, and the monomeric composition of polysaccharides.

Ash was determined by measuring the residue remaining after incinerating the sample overnight in a muffle furnace at 525 °C.[26] The extractives were determined with procedures adapted from TAPPI 204 cm-97, in a Soxhlet system successively with dichloromethane (6 h), ethanol (16 h), and water (16 h). The extractives solubilized by each solvent were determined by mass difference of the solid residue after drying at 105 °C and reported as percent of the original sample.

The suberin content was determined in the extractive-free material by use of methanolysis for depolymerization.[27] A 1.5 g sample of extractive-free material was refluxed with a 3% (m/v) solution of $NaOCH_3$ in CH_3OH (100 ml) during 3 h. The sample was filtrated and washed with methanol and the residue refluxed again with 100 ml CH_3OH for 15 min and filtrated. The combined filtrates were acidified to pH 6 with 2 M H_2SO_4 and evaporated to dryness. The residues were suspended in water (50 ml) and the products recovered with dichloromethane in three successive extractions (of 50 ml each). The combined extracts were dried over anhydrous Na_2SO_4, and the solvent evaporated to dryness. The suberin extracts were quantified and the results expressed in percent of the initial dry mass.

Klason and acid-soluble lignin, and carbohydrates contents were determined on the extracted and desuberinized materials. Sulfuric acid (72%, 3.0 ml) was added to 0.35 g of the sample and the mixture placed in a water bath at 30 °C for 1 h. The solution was diluted with water until the sulfuric acid concentration was 3% and then autoclaved at 121 °C for 30 min. After cooling, the insoluble fraction was separated by filtration and the Klason lignin was weighed after drying at 105 °C [28] and the acid-soluble lignin determined by measuring the UV absorption at 206 nm using an extinction coefficient of 110 l g^{-1} cm^{-1}.[29] The remaining acid solution was kept for sugar monomer analysis.

The composition of polysaccharides was evaluated by determining the content in neutral monossacharides (rhamnose, arabinose, xylose, galactose, mannose, and glucose) and uronic acids (galacturonic and glucuronic acids) in the hydrolysate from the lignin analysis using High Pressure Ion-exchange Chromatography with a pulsed amperometric detector (HPIC-PAD). The compounds were separated in a Dionex ICS-3000 system, with an Aminotrap plus Carbopac PA10 column (250 × 4 mm). The content of acetic acid (acetyl groups are substitution units in monosaccharides) was also determined in the hydrolysate using a High-Pressure Ion-exclusion Chromatography with a UV/Visible detector (HIPCE-UV). The compounds were separated in a Thermo Finnigan Surveyor installed with a Biorad Aminex 87 H column (300 × 7.8 mm).

Suberin Composition

Aliquots of the dichloromethane extracts (5 ml) from the suberin depolymerization reaction were taken, evaporated under N_2 flow and dried at room temperature under vacuum overnight. The samples were derivatized prior to analysis: they were dissolved in 120 µl of pyridine and the compounds with hydroxyl and carboxyl groups were trimethylsilylated into trimethylsilyl (TMS) ethers and esters, respectively, by adding 80 µl of bis-(trimethylsily)-trifluoroacetamide (BSTFA). The reaction mixture was heated at 60 °C for 30 min in an oven and immediately analyzed by injection in a GC–MS Agilent 5973 MSD with the following GC conditions: Zebron 7HG-G015-02 column (30 m, 0.25 mm; ID, 0.1 µm film thickness), constant flow of 1 ml min^{-1}, front injector equipped with a SS inlet

liner He packed with wool (dimensions 1.5 mm ID ×78.5 mm L × 6.45 mm OD) at 280 °C in a splitless mode, oven temperature program, 100 °C (1 min), rate of 8 °C min^{-1} up to 250 °C, rate of 5 °C min^{-1} up to 300 °C (5 min), rate of 5 °C min^{-1} up to 350 °C (5 min), rate of 10 °C min^{-1} up to 380 °C (5 min). In the MS analyzer (Triple axis Detector) the source was kept at 220 °C (with the transfer line temperature at 320.2 °C) and the electron impact mass spectra (EIMS) taken at 70 eV of energy. The compounds were identified and quantified as TMS derivatives by comparing their mass spectra with a GC–MS spectral library (Wiley, NIST), and by comparing their fragmentation profiles with published data, reference compounds, ion fragmentation patterns, and/or retention times. Each aliquot was injected in triplicate and results presented by mean (only standard deviation inferior to 5% was considered).

RESULTS AND DISCUSSION

The *P. menziesii* trees showed a conspicuous outer bark which included at the stem base a thick rhytidome with several cork-rich periderms interspersed by phloem layers while at younger ages in the upper stem height levels it included only one periderm. The age-related structural variation of the bark is exemplified in Figure 1, representing cross-sectional images of the bark at the base, middle and top of the stem of one tree that corresponded to cambial ages of respectively 45, 30, and 17 years. It is clear that the striking difference is the development of several periderms in the rhytidome with age, and thereby the presence of cork in substantial amounts in the older stages.

Chemical Composition of Bark

Table 1 summarizes the chemical composition of the Douglas-fir bark at different ages, as given by the sampling taken at three stem height levels in both sites. The bark structural variation along the stem (as exemplified in Figure 1) leads to chemical differences between the different height levels.

At stem base, Douglas-fir bark shows a high content of extractives ranging from 27.5% (site Estrela) to 31.2% (site Cabreira). Polar compounds extracted by ethanol and water represented approximately 80% of the total extractives (corresponding to 21.7–25.2% of the bark). The main cell wall structural component of the bark at stem base is suberin (23.1% in Cabreira and 27.7%

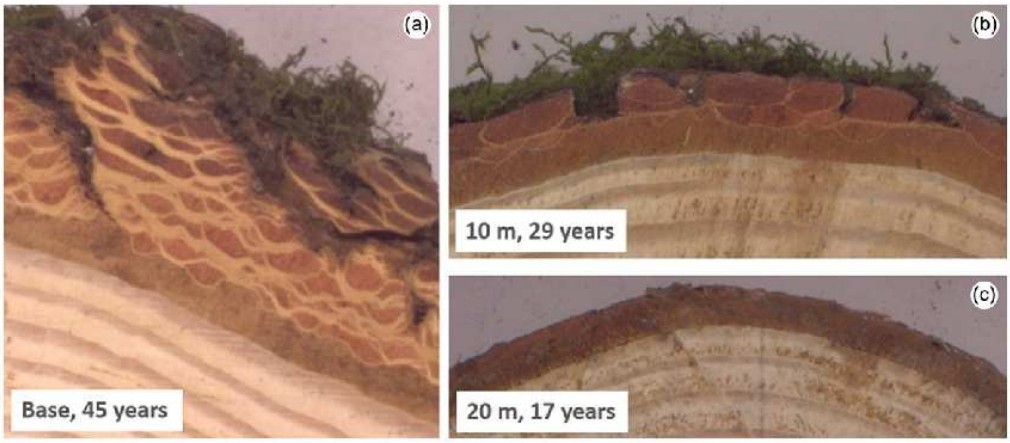

FIGURE 1. Cross-sectional images of the bark of one *Pseudotsuga menziesii* tree at three stem height levels: (a) base, corresponding to a cambial age of 45 years, with 6 mm phloem and 31 mm rhytidome, containing six periderms; (b) 10 m, corresponding to a cambial age of 29 years, with 6 mm phloem and 12 mm rhytidome, containing two periderms; and (c) 20 m, corresponding to a cambial age of 17 years, with 7 mm phloem and without rhytidome.

TABLE 1. Chemical composition (% of o.d. mass) of Douglas-fir bark taken from three trees in two sites (Cabreira and Estrela) at three levels of stem height: base, middle (10 m) and top (20 m).

	Cabreira			Estrela		
	Base	Middle	Top	Base	Middle	Top
Ash	0.7 ± 0.1	1.2 ± 0.36	1.7 ± 0.3	0.7 ± 0.1	1.4 ± 0.4	1.6 ± 0.7
Extractives total	31.2 ± 5.1	27.7 ± 3.6	32.1 ± 5.8	27.5 ± 5.7	13.5 ± 1.5	19.3 ± 1.9
Dichloromethane	6.1 ± 0.2	1.0 ± 0.8	3.3 ± 2.3	5.8 ± 0.2	2.5 ± 1.4	4.9 ± 4.2
Ethanol	23.0 ± 5.2	21.4 ± 5.1	22.6 ± 4.7	19.2 ± 5.8	5.3 ± 1.3	7.8 ± 2.3
Water	2.2 ± 0.2	5.2 ± 1.9	6.2 ± 2.1	2.5 ± 0.5	5.8 ± 0.5	6.9 ± 0.9
Suberin	23.1 ± 1.6	2.4 ± 1.1	0.0 ± 0.0	27.7 ± 5.4	2.8 ± 1.3	1.8 ± 0.0
Lignin total	25.5 ± 2.3	31.5 ± 4.1	26.7 ± 3.4	24.7 ± 2.1	36.8 ± 3.6	32.9 ± 1.6
Klason lignin	24.9 ± 2.3	30.4 ± 3.9	26.0 ± 3.6	24.1 ± 2.1	35.6 ± 3.3	32.9 ± 1.7
Soluble lignin	0.6 ± 0.1	1.1 ± 0.3	0.7 ± 0.3	0.5 ± 0.1	1.2 ± 0.4	1.1 ± 0.1
Polysaccharides[a]	19.5 ± 2.6	37.2 ± 2.2	±12.7	19.4 ± 4.4	45.5 ± 3.6	44.2 ± 2.4

Mean and standard deviation.
[a]By difference.

in Estrela) but almost in the same proportion as Klason lignin (24.5% on average); the average suberin:lignin ratio was 1.0. These results are in direct relation with the structure of the Douglas-fir bark at this height level: presence of a rhytidome with a large proportion of cork (the suberin-containing tissue) in the lower part of the stem (Figure 1).

Polysaccharides (Table 2) are mainly constituted by glucose (63.6% of the total monosaccharides) and a significant content of arabinose (10.8%). The content of galactose and mannose represented 15.2%, and of arabinose and xylose 16.5% of the total monosaccharides, suggesting that the main hemicelluloses are galactoglucomannans and arabinoxylans. Galacturonic acid was present (3.5%) but not glucuronic acid nor acetyl groups.

The chemical composition reported here is consistent with that given previously for Douglas-fir bark[19]: total extractives 20.4% (of which 16.0% ethanol extractives), suberin 25.6%, lignin 27.7% and polysaccharides 26.3% with similar composition (glucose represents 65.7% of all monosaccharides, arabinose 7.1%, xylose 9.0%, and mannose 11.8%).

There are not many published references on the chemical composition of barks. In general, barks have high content of extractives and lignin, while the suberin content varies largely depending on the extent of the presence of cork.[1,23] Barks with a small proportion of cork have a low content of suberin: *Pinus sylvestris* bark contains 21.6% total extractives, 1.3% suberin, and 27.9% lignin; *Picea abies* contains bark 18.8% total extractives, 1.6% suberin, and 33.7% lignin[30]; *Pinus pinaster* bark contains 11.4% total extractives, 1.5% suberin, and 43.7% lignin[31]; and *Pinus pinea* bark contains 19.1% total extractives, 2.5% suberin, and 43.5% lignin.[31] On the contrary, barks with higher proportion of cork have more suberin, as expected, for example, *B. pendula* bark contains 17.6% total extractives, 5.9% suberin and 27.9% lignin.[11]

Age-Variation of Chemical Composition

There was a significant chemical variation between the barks with different ages (Table 1). Extractives content was highest in the oldest barks at the stem base, decreasing to the middle and then increasing to the top (on average 29.4%, 20.6%, and 25.7%, respectively). The increase in bark extractives in the upper part of the tree may be explained by the proximity to the crown with more translocation of metabolic compounds. Although the axial variation pattern of extractives was similar in all the trees, the extent of age-related variation differed between sites: the bark of Estrela trees had less extractives than that of Cabreira trees, for example, 19.3% and 32.1% at the top level respectively (Table 1).

TABLE 2. Composition of polysaccharides (% of total units) determined after acid hydrolysis as neutral monosaccharides, uronic acids and acetic acid of Douglas-fir bark taken from three trees in two sites (Cabreira and Estrela) at three levels of stem height: base, middle (10 m) and top (20 m).

	Cabreira			Estrela		
	Base	Middle	Top	Base	Middle	Top
Percentage of total sugars						
Rhamnose	1.3 ± 0.1	1.2 ± 0.3	1.0 ± 0.7	1.0 ± 0.3	1.0 ± 0.1	1.3 ± 0.3
Arabinose	11.0 ± 0.6	11.0 ± 1.3	12.1 ± 2.0	10.5 ± 2.1	9.9 ± 1.1	10.3 ± 2.4
Galactose	6.8 ± 0.5	6.5 ± 0.4	6.3 ± 0.7	6.0 ± 0.9	6.1 ± 0.5	6.4 ± 0.7
Glucose	63.0 ± 0.9	63.3 ± 1.7	61.7 ± 5.3	64.3 ± 2.7	69.9 ± 2.1	69.0 ± 6.4
Xylose	5.7 ± 0.5	5.6 ± 0.8	6.3 ± 5.1	5.8 ± 0.7	3.8 ± 0.1	4.3 ± 2.8
Mannose	8.8 ± 0.2	8.8 ± 0.9	8.7 ± 4.0	8.9 ± 1.5	5.8 ± 0.3	6.8 ± 3.2
Galacturonic acid	3.6 ± 0.5	3.8 ± 0.5	3.0 ± 1.4	3.5 ± 1.6	3.6 ± 0.3	1.6 ± 2.1
Glucuronic acid	0.0 ± 0.0	0.5 ± 0.03	0.5 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Acetic acid	0.0 ± 0.0	0.2 ± 0.2	0.4 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 1.6

Mean and standard deviation.

The most striking chemical effect related with age was the content in suberin (Table 1). While the bark at stem base contained a considerable amount of suberin, representing on average 25.4% of the bark, the bark in the upper part of the stem contained a very small amount of suberin (less than 2% in Estrela and no suberin at all in the barks at Cabreira). In the mid-section of the stem, at 10 m of height, suberin is present in the bark with an average content of 2.6%. These differences in suberin content are in direct relation with the differences in the structure of the barks at the three height levels (Figure 1), that is, with the absence of a rhytidome with suberized phellem tissues in the young barks. The cork proportion in the outer bark is about 50% at the stem base while in the upper portions of the stem the bark has not yet formed a rhytidome.[19]

The analysis of the age-related lignification of the bark is more easily followed if the lignin content is expressed on extractive-free and desuberized bark material (Figure 2). Cell wall lignification increased with cambial age, that is, lignin content was highest at stem base and was similar in both sites. This agrees with the age-induced changes in the phloem with increasing cell sclerefication of the collapsed phloem. Such anatomical changes were also shown in the bark of *Q. cerris*.[8]

On the contrary, there were no significant aging effects on the monomeric composition of polysaccharides (Table 2).

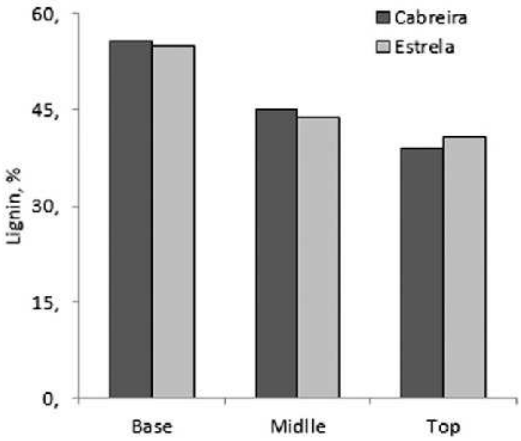

FIGURE 2. Lignin content of *Pseudotsuga menziesii* barks at three stem height levels (base, middle, top) calculated on extractive-free and suberin-free barks.

Chemical Composition of Cork and Phloem

The cork and phloem fractions were carefully separated from the rhytidome at the stem base of two trees and were analyzed individually (Table 3). There are very clear chemical differences between these two bark components.

Douglas-fir cork has a very high content of extractives (on average 49.8%) of which 8.2% are non-polar compounds and 41.6% are polar compounds soluble in ethanol and water (corresponding to 82% of the total extractives). The suberin content is also high,

representing on average 30.1% of the Douglas-fir cork. The lignin and polysaccharides contents are low, respectively 11.4% and 8.7%. Ash content is also very low (0.3%).

The chemical composition found here is similar to the values reported for the cork of Douglas-fir: 29.2% and 37.5% of extractives and 36% and 33% of suberin.[18,20] Douglas-fir cork contains a higher amount of extractives as compared to the average values reported for the corks of other tree species: *Q. suber* (16.2%),[32] *Q. cerris* (16.7%),[8] *Q. variabilis* (9.6%),[11] and *Kielmeyera coriacea* (15–20%).[33,34]

The composition of extractives, characterized by a higher content of the polar fraction in relation to the lipophilic fraction (Table 3), was observed also for *Q. suber* cork (64% of the total extractives[32]). However, this is contrary to what happens in corks of other species where the fraction of lipophilic compounds is higher, for example, 50% of total extractives in *K. coriacea*,[33] 51% in *Q. variabilis*,[11] and 65% in *Q. cerris*.[8]

The suberin content, when determined on an extractive-free basis (corresponding to 59.7%) is within the natural variability range found for *Q. suber* cork (35–57%)[27,35,36] and comparable to that of *Q. variabilis* cork (43.3–50%)[11,37] and *Q. cerris* cork (34.2%),[8] but higher than the 21–35% reported for *K. coriacea* cork.[33,34]

The lignin content (22.1% on an extractive-free basis) is within the range found for *Q. suber* cork (17–35% of extractive-free cork),[27,32] but lower than in the cork of *Q. cerris* (33.7% of extractive-free cork),[8] and of *Q. variabilis* (24.5% of extractive-free cork).[30] The suberin:lignin ratio is 2.6, a value similar to the one in *Q. suber* cork.[32]

The carbohydrate composition (Table 4) shows a predominance of glucose (on average 44.3% of the total monosaccharides), a substantial amount of arabinose and xylose (34.1% of the total monosaccharides), and galactose and mannose (15.4%) including uronic acid monomers especially galacturonic acid (3.7%) and a few acetyl substitutions.

The main hemicelluloses are therefore arabinoxylans and galactoglucomannans. This composition is very similar to that reported previously for Douglas-fir cork[20] of 55.4% glucose, 13.3% xylose, 10.9% arabinose, 10.3% galactose, and 10.1% mannose. It is also similar to that of other corks in relation to the predominance of glucose and a substantial xylose and arabinose content.[2]

As regards phloem, its composition reflects its lignocellulosic nature and clearly differs from that of cork (Table 3): a high lignin content, ranging 35.0–38.2% (44.0% of extractive-free phloem) and also a high extractives content (13.8 and 20.1% of the initial dry mass, respectively in Estrela and

TABLE 3. Chemical composition (% of o.d. mass) of cork and phloem fractions separated from the rhytidome of Douglas-fir bark at the base of trees in two sites (Cabreira and Estrela).

	Cabreira		Estrela	
	Phloem	Cork	Phloem	Cork
Ash	1.4 ± 0.6	0.2	2.1 ± 0.4	0.3
Extractives total	20.1 ± 7.0	53.1	13.8 ± 0.5	46.5
Dichloromethane	1.0 ± 0.4	9.0	1.6 ± 1.4	7.3
Ethanol	15.1 ± 7.3	36.3	5.5 ± 1.5	36.4
Water	4.0 ± 0.5	7.8	6.7 ± 0.5	2.7
Suberin	–	26.1	–	34.1
Lignin total	35.0 ± 6.5	10.8	38.2 ± 4.546	12.1
Klason lignin	34.3 ± 6.5	10.4	37.3 ± 4.5	11.8
Soluble lignin	0.7 ± 0.1	0.4	0.9 ± 0.2	0.2
Polysaccharides[a]	43.3 ± 3.2	10.0	48.1 ± 5.0	7.3

For phloem mean of three trees and standard deviation.
[a]By difference.

TABLE 4. Composition of polysaccharides (% of total units) determined after acid hydrolysis as neutral monosaccharides, uronic acids and acetic acid of cork and phloem separated from the rhytidome of Douglas-fir bark at the base of trees in two sites (Cabreira and Estrela).

	Cabreira		Estrela	
	Phloem	Cork	Phloem	Cork
Rhamnose	0.0 ± 0.0	1.9	0.7 ± 0.1	2.2
Arabinose	5.4 ± 0.74	23.3	4.2 ± 0.6	27.3
Galactose	4.5 ± 0.60	8.3	3.9 ± 0.2	10.4
Glucose	69.5 ± 3.02	45.5	71.4 ± 3.9	43.1
Xylose	7.6 ± 0.86	8.4	5.4 ± 1.2	9.2
Mannose	6.3 ± 3.97	7.0	7.7 ± 1.8	3.7
Galacturonic acid	4.5 ± 0.62	4.3	4.3 ± 0.3	3.0
Glucuronic acid	0.7 ± 0.08	0.8	0.0 ± 0.0	0.8
Acetic acid	1.5 ± 0.47	0.3	2.6 ± 0.4	0.3

For phloem mean of three trees and standard deviation.

Cabreira). The ethanol and water extracts were mainly responsible for this high extractive content (12.2 and 19.1% of the initial dry mass, respectively in Estrela and Cabreira). The carbohydrate composition is dominated by glucose (70.5% of total sugars) with xylose and arabinose representing together 11.5% of the total monosaccharides, and mannose and galactose 11.2% (Table 4).

These values are consistent with the 28.4% total extractives, 35.1% lignin and polysaccharide composition reported for Douglas-fir phloem.[20] A similar lignin content (35%) is also reported for the phloem of *Q. cerris* bark but with less content of extractives (6.5%) and a different carbohydrate composition (glucose 47.2%, xylose and arabinose 47%, mannose and galactose 4.8%).[8]

These chemical differences between cork and phloem clearly explain the age effect that was found on the bark chemical composition, as previously described (Table 1).

Variation of Suberin Composition

In the suberin depolymerization extracts of *P. menziesii* cork it was possible to identify most of the peaks (93.9% and 87.1% of total compounds in Cabreira and Estrela respectively), as shown in Table 5 for the analysis of cork from the stem base. Suberin is mainly constituted (Table 5) on average by α,ω-alkanoic diacids with 27.6% of all compounds (26.7% saturated and 1.3% substituted α,ω-alkanoic diacids only in Estrela tree samples), ω-hydroxyalkanoic acids with 35.2% (31.7% saturated and 3.5% substituted ω-hydroxyalkanoic acids), and alkanoic acids with 18.1% (9.1% saturated and 9.0% substituted alkanoic acids). Alkanols are present (2.2%) as well as aromatics (8.8%).

Previous reports on suberin composition of *P. menziesii* cork indicated overall similarities although with some differences in relation to the present values: for instance, the suberin showed higher content in α,ω-alkanoic diacids and lower in ω-hydroxyalkanoic acids and alkanoic acids (53.9%, 16.9%, and 8.0%, respectively, without considering glycerol).[18]

TABLE 5. Composition of suberin from cork from *Pseudotsuga menziesii* tree barks at the stem base from Serra da Cabreira and Serra da Estrela, in % of the chromatographic peak areas of the compounds detected in GC-MS (for simplification free and methyl ester forms are grouped).

Identified compounds	Cabreira	Estrela
Aromatics	***8.44***	***9.15***
Vanillin	0.64	0.62
Vanillic acid	0.42	0.41
Benzoic acid	–	0.33
3,4-Dihydroxybenzoic acid	0.74	0.88
4-Methoxybenzoic acid	–	0.24
3-Hydroxybenzoic acid	–	0.22
3-Hydroxy-3-methoxy-2-propenoic acid	–	0.48
Ferulic acid	6.64	5.97
Alkanols	***1.95***	***2.34***
4-Methyl-3-heptanol	–	0.21
Ethylene glycol	–	0.56
Octanol	–	0.20
Pentadecanol	–	0.16
Hexadecanol	–	0.09
Eicosanol	0.22	–
Docosanol	1.21	0.77
Tetracosanol	0.52	0.35
Saturated alkanoic acids	***9.59***	***8.66***
Hexanoic acid	0.26	–
Heptanoic acid	–	0.14
Octanoic acid	0.25	0.21
Decanoic acid	0.44	
Hexadecanoic acid	0.22	0.34
Eicosanoic acid	0.91	0.92
Docosanoic acid	4.17	3.46
Tetracosanoic acid	3.00	3.33
Hexacosanoic acid	0.34	0.26
Substituted alkanoic acids	***12.23***	***5.77***
Dec-9-enoic acid	4.83	4.51
18-Hydroxyoctadecanoic acid	1.57	0.13
9,12-Octadecadienoic acid	5.62	–
Octadec-9-enoic acid	–	0.15
8,9,18-Trihydroxyoctadecanoic acid	–	0.51
9,10,18-Trihydroxyoctadecanoic acid	0.24	0.47
Saturated α,ω-alkanoic diacids	***24.97***	***28.39***
Pentanedioic acid	0.34	0.39
Hexanedioic acid	2.34	2.63
Heptanedioic acid	0.24	0.07
Octanedioic acid	1.23	1.44
Nonadioic acid	1.70	1.67
Hexadecanedioic acid	10.05	11.11
Octadecanedioic acid	5.36	8.69
Eicosanedioic acid	1.77	1.29
Docosanedioic acid	0.76	1.10
Tetracosanoic acid	1.18	–
Substituted α,ω-alkanoic diacids	–	***1.34***
9,10-Dihydroxyoctadecanedioic acid	–	1.34
Saturated ω-hydroxyalkanoic acids	***31.22***	***32.22***
6-Hydroxyhexanoic acid	0.72	0.85
8-Hydroxyoctanoic acid	0.09	–
9-Hydroxynonanoic acid	0.58	0.59
10-Hydroxydecanoic acid	–	0.59
16-Hydroxyhexadecanoic acid	20.01	21.56

(*Continued*)

TABLE 5. (*Continued*).

Identified compounds	Cabreira	Estrela
18-Hydroxyoctadecanoic acid	4.18	4.26
8,9,18-trihydroxyoctadecanoic acid	0.40	–
20-Hydroxyeicosanoic acid	1.45	4.37
22-Hydroxydocosanoic acid	3.31	–
24-Hydroxytetracosanoic acid	0.39	–
Substituted ω-hydroxyalkanoic acids	***2.40***	***4.55***
9,10-Epoxy-18-hydroxyoctadecanoic acid	–	0.33
18-Hydroxy-9-octadecenoic acid	–	4.22
22-Hydroxydocosanoic acid	2.40	–
Glycerol	***0.20***	***0.43***
Glycerol	0.20	0.43
Identified	**90.90**	**87.09**
Non-identified	9.10	12.91
Total	100	100

Also higher contents in ω-hydroxyalkanoic acids and lower contents in α,ω-alkanoic diacids and alkanoic acids (36.2%, 18.6% and 6.2%, respectively) are reported but the level of compound identification was under that found here (only 75% of the compounds were identified).[20] Another difference lies on the absence of sterols in the suberin extract.[20]

As regards single compounds (Table 5), the major suberin monomers are the 16-hydroxyhexadecanoic acid with 20.8% and the hexadecanedioic acid with 10.6% with also important amounts of octadecanedioic acid with 7.0%, 18-hydroxyoctadecanoic acid with 4.2% and docosanoic acid with 3.8%. These are also important suberin monomers that were reported previously.[18,20]

Ferulic acid is present in the suberin extract, corresponding to 6.3% (Table 5). Ferulic acid is commonly found in suberin monomer mixtures in proportion less than 1%[38] or up to 9% under more aggressive depolymerization conditions.[39,40] Ferulic acid acts as a bridging monomer between suberin and lignin.[41]

These results for cork suberin composition are in line with the composition of suberin from other species as regards the presence of the main chemical families, although their proportion varies with species.[2,23] For instance, in cork from *Q. suber* trees from Bulgaria and Turkey ω-hydroxyalkanoic acids are the most abundant compounds (20.7%–33.0%), with substituted alkanoic acids (13.4%–14.4%) and substituted α,ω-alkanoic diacids (8.9%–15.3%) also in considerable amounts.[42] The suberin of *P. reticulata* cork[15] is also mainly constituted by saturated ω-hydroxyalkanoic acids (40.7%) and saturated α,ω-alkanoic diacids (21.4%), and substituted α,ω-alkanoic diacids, alkanoic acids and substituted ω-hydroxyalkanoic acids were also identified in amounts varying around 8.0% and 8.7%. In *B. pendula*, saturated ω-hydroxyalkanoic acids constitute 55.2%–83.9% of all the compounds identified in suberin extracts.[13,14] In *Q. variabilis,* the suberin extract is mainly constituted by ω-hydroxyalkanoic acids (27.0%–32.1%) and

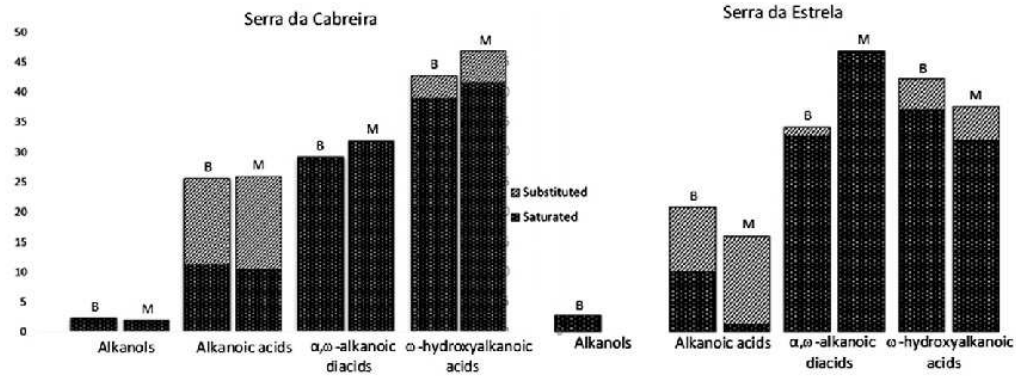

FIGURE 3. Comparison of suberin composition in the bark at stem base (B) and mid-height (M) of *Pseudotsuga menziesii* trees collected in Cabreira and Estrela: proportion of the chemical families of alkanols, alkanoic acids, α,ω-alkanoic diacids and ω-hydroxyalkanoic acids (saturated and substituted) calculated in % of the identified long-chain lipid compounds.

substituted α,ω-alkanoic diacids (12.1%–26.9%).[12]

When analyzing the variation of the suberin composition by comparing trees from both sites (Table 5), it was found that the differences were not substantial, except for instance in substituted alkanoic acids that represent 12.2% and 5.8% or substituted ω-hydroxyalkanoic acids representing 2.4% and 4.6% in Cabreira and Estrela, respectively.

As regards the effect of age on the suberin composition, Figure 3 shows the comparison between the contents of chemical families (in % of the identified long-chain lipid compounds) determined at two cambial ages: approximately 45 years at stem base level and 30 years at mid-height. For the trees at Cabreira no differences were found. For the trees at Estrela there were some compositional differences although not very high: in the older bark at stem base and in comparison with the younger bark at mid-stem, the suberin showed a decrease in saturated alkanoic acids (8.7% vs. 0.7%) and in saturated ω-hydroxyalkanoic acids (32.2% vs. 19.6%).

CONCLUSIONS

Douglas-fir bark shows a high content of polar extractives at all stem height levels that constitute a potential valorization route for the whole bark. Extractives, namely polyphenols such as flavonoids or tannins, are present in both phloem and cork tissues, and therefore their fractionation and valorization may also be considered when phloem and cork streams are processed separately. Phloem-enriched fractions can go subsequently through further processing to produce a lignin-pure material.

Regarding suberin content, there is a clear age-related chemical variation as a consequence of the structural variation of bark with age with formation of successive periderms and their cork layers. Cork, and consequently suberin, contents are small in bark with less than 30 years of age. Therefore a valorization of Douglas-fir bark targeting cork should use the lower stem parts of mature trees, that is, the bottom logs of trees harvested for sawmills. The cork fractionation process includes bark milling, followed by granulometric and densimetric fraction separation. Similar processes were already tested for separation of cork from *Q. cerris*[8] and proposed for *B. pendula*.[13] The valorization of the cork component may target either the production of cork-based products or of chemicals such as extraction of terpenoids, production of long-chain fatty acids, and the production of mono and oligosaccharides.

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Conclusions and future work

The research carried out along this PhD project allowed to obtain new knowledge on the stem development of Douglas-fir trees grown in Portugal and their bark structural and chemical characterization in view of its potential utilization through the valorization of the cork component.

Considering the specific objectives of the research project, the conclusions can be summarized as follows:

a) Douglas-fir trees contain a substantial proportion of heartwood that decreased from tree base upwards, i.e. decrease with decreasing of tree age, and that is estimated to start early at 8–9 years of cambial age and increasing by 0.7–0.9 rings year^{-1}. Sapwood width was on average 75 mm with small axial variation, i.e. near constant along tree height. The bark content is high especially in the lower part of the stem, representing 12 % of the stem volume and decreasing with tree height and tree age.

b) Douglas-fir has a thick bark and develops with age a rhytidome with several periderms that contain a substantial proportion of cork. The proportion of cork in the bark increases with age and becomes interesting from a yield perspective for mature trees and older cambial ages, namely a 50 % cork content in the rhytidome at 50 years of cambial age. Bark valorization is therefore advantageous to be included within the Douglas-fir timber exploitation and to consider the species as a potential cork-provider for the cork industry. The integration of the Douglas-fir bark valorization in the current exploitation for sawmill processing means that it will be the lower part of the stem e.g. the logs up to 5 m of height (where bark thickness is highest) that should be directed for debarking and bark processing to recover the cork component.

c) The rhytidome of Douglas-fir bark has a substantial amount of cork that appear as thin layers that are spatially discontinuous and interspersed by phloem. The utilization of the cork material will require a trituration process of the bark and a fractionation to separate pure cork fractions. Therefore, cork will be obtained from the Douglas-fir bark only as a granulated material that can be processed into agglomerates. The striking feature of Douglas-fir cork is the presence of

bands of crushed or heavily corrugated cells that are radially compressed, making up a compressed and very compact structure with patches of uncompressed cork. The use of Douglas-fir cork as a cellular material with properties like those of commercial cork from *Quercus suber* is therefore not possible without cell expansion and straightening of cell walls.

d) The chemical composition of Douglas-fir bark shows a very high content of polar extractives at all stem height levels that constitute a potential valorization route for the whole bark. The high extractives content is present in both phloem and cork. There is a clear age-related chemical variation of the bark regarding suberin content: suberin content is low in the higher parts of the stem and increases to the base of the trees, where it is highest. The suberin content is in direct relation with the proportion of cork in the bark since phloem does not contain suberin. Therefore the chemical variation is a consequence of the structural variation of bark with age by formation of successive periderms and cork layers. Cork and consequently suberin contents are small in bark with less than 30 years of age but increase significantly at older cambial ages. A valorization targeting cork should therefore use the lower stem parts of mature trees harvested for sawmill processing.

Despite the need for further studies, the results suggest that the bark of mature trees of Douglas-fir over 35 years of age could be considered as a source of cork although the separation of pure fractions of cork require fractioning given the presence in the rhytidome of Douglas-fir bark of only thin layers of cork interspersed by phloem. The results found in this thesis and the conclusions presented here suggest that further research is needed, namely on the following subjects:

- The fractionation of the Douglas-fir bark in view of the separation of the cork component as fractions with different granulometries with a high degree of purity, based on the difference of properties (in particular density and permeability) of the different anatomical components of the bark should be investigated. This point is important to enable an industrial use of cork.

- Apart from lab-scale fractionation, a pilot scale trituration and separation should be carried out in order to obtain demonstrative results on potential industrial yields e.g. yield of cork-only and cork-rich granulates, and to obtain quantities allowing further application studies, namely product development.

- The properties of the Douglas-fir cork granulated material e.g. density and bulk density, wettability and gluing properties should be investigated and compared with the granulated material from *Quercus suber* cork.

- Given the presence of extensive areas of radially crushed or heavily corrugated cork cells, the capacity of cell expansion and straightening of cell walls of cork of Douglas-fir bark should be investigated.

- The application of the Douglas-fir cork granulates to make agglomerated cork stoppers and other agglomerates should be studied and the product properties analyzed.

- The use of the phloem component as a feedstock in a biorefinery perspective should be studied considering different valorization routes e.g. extractives for bioactive compounds and for green chemicals, biofuels and composite materials.

- The economic feasibility of Douglas-fir bark utilization should also be addressed once product yields and performance are known.

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Appendixes

Appendix I – Location of disc samples along the tree height, and characterization in terms of age (number of rings), and number of rings of heartwood and sapwood for each tree in the sites of Cabreira and Estrela.

Site: Cabreira

					Number of annual rings			
Tree nº	Disc nº	Tree height (m)	Age (nº of rings)		Heartwood	Sapwood	Heartwood	Sapwood
1	1	0.3	45	46	34	11	34	12
	2	3.1	38	38	26	12	26	12
	3	5.7	36	36	21	15	21	15
	4	8.3	33	33	20	13	21	12
	5	10.9	31	31	18	13	19	12
	6	13.6	24	24	12	12	12	12
	7	16.2	22	22	11	11	11	11
	8	18.8	19	19	9	10	9	10
	9	21.5	15	15	6	9	6	9
	10	24.1	11	11	3	8	3	8
	11	26.7	8	7	0	8	0	7
2	1	0.1	46	46	37	9	37	9
	2	2.8	38	38	25	13	27	11
	3	5.4	35	35	22	13	22	13
	4	8.1	33	33	21	12	22	11
	5	10.7	30	30	18	12	19	11
	6	13.3	28	28	18	10	18	10
	7	16.0	25	25	15	10	15	10
	8	18.6	22	22	13	9	13	9
	9	21.2	19	19	11	8	11	8
	10	23.8	14	14	7	7	7	7
	11	26.5	10	10	4	6	4	6
	12	29.1	6	6	0	6	0	6
3	1	0.2	44	45	26	18	29	16
	2	2.9	40	40	21	19	21	19
	3	5.5	36	36	18	18	18	18
	4	8.1	33	33	15	18	16	17
	5	10.8	29	29	13	16	13	16
	6	13.4	25	25	10	15	11	14
	7	16.0	21	21	8	13	8	13
	8	18.6	17	17	6	11	6	11
	9	21.2	12	12	2	10	2	10
	10	23.9	9	9	0	9	0	9
	11	26.4	6	6	0	6	0	6
4	1	0.2	43	42	32	11	30	12
	2	2.8	36	36	24	12	23	13
	3	5.5	33	33	20	13	21	12
	4	8.1	30	30	18	12	19	11
	5	10.7	27	27	16	11	16	11
	6	13.3	24	24	13	11	14	10
	7	15.9	19	19	10	9	9	10
	8	18.5	16	16	7	9	8	8
	9	21.1	12	12	4	8	4	8

Site: Cabreira

					Number of annual rings			
Tree nº	Disc nº	Tree height (m)	Age (nº of rings)		Heartwood	Sapwood	Heartwood	Sapwood
5	1	0.1	45	46	44	1	41	5
	2	2.8	38	38	29	9	27	11
	3	5.4	34	34	23	11	23	11
	4	8.0	31	31	20	11	20	11
	5	10.6	28	28	17	11	18	10
	6	13.2	24	24	14	10	14	10
	7	15.8	21	21	12	9	12	9
	8	18.4	16	16	8	8	8	8
	9	21.0	13	13	6	7	5	8
	10	23.6	10	10	3	7	3	7
	11	26.2	6	6	0	6	0	6
6	1	0.1	43	44	32	11	33	11
	2	2.8	36	36	25	11	25	11
	3	5.4	30	30	20	10	20	10
	4	8.0	25	25	17	8	16	9
	5	10.7	21	21	13	8	13	8
	6	13.3	17	17	9	8	9	8
	7	15.9	14	14	8	6	8	6
	8	18.5	11	11	4	7	4	7
	9	21.1	7	7	1	6	1	6
7	1	0.2	50	50	36	14	38	12
	2	2.8	39	39	26	13	27	12
	3	5.5	36	36	23	13	23	13
	4	8.1	33	33	22	11	22	11
	5	10.7	30	29	20	10	19	10
	6	13.4	26	26	16	10	16	10
	7	16.0	22	22	13	9	13	9
	8	18.6	18	18	11	7	11	7
	9	21.2	13	13	7	6	7	6
	10	23.8	10	10	4	6	4	6
8	1	0.2	45	45	37	8	37	8
	2	2.9	36	36	25	11	25	11
	3	5.5	34	34	23	11	23	11
	4	8.2	31	31	20	11	21	10
	5	10.8	28	28	17	11	19	9
	6	13.4	25	25	15	10	15	10
	7	16.0	20	20	11	9	11	9
	8	18.7	17	17	9	8	9	8
	9	21.3	14	14	7	7	6	8
	10	23.9	10	10	4	6	3	7

Site: Cabreira

					Number of annual rings			
Tree nº	Disc nº	Tree height (m)	Age (nº of rings)		Heartwood	Sapwood	Heartwood	Sapwood
9	1	0.2	44	44	34	10	34	10
	2	2.8	37	37	27	10	27	10
	3	5.4	33	33	24	9	24	9
	4	8.0	30	30	21	9	22	8
	5	10.6	26	26	18	8	17	9
	6	13.2	23	23	15	8	15	8
	7	15.8	18	18	11	7	11	7
	8	18.4	14	14	8	6	8	6
	9	21.0	11	11	6	5	6	5
	10	23.6	8	8	3	5	3	5
10	1	0.2	47	48	31	16	33	15
	2	2.9	38	38	22	16	21	17
	3	5.6	36	36	20	16	20	16
	4	8.2	31	31	18	13	18	13
	5	10.9	29	29	18	11	16	13
	6	13.5	25	25	15	10	14	11
	7	16.2	22	22	11	11	11	11
	8	18.8	18	18	8	10	8	10
	9	21.4	16	16	8	8	8	8
	10	24.0	12	12	5	7	4	8
	11	26.7	10	10	1	9	1	9
	12	29.3	7	7	0	7	0	7

Site: Estrela

					Number of annual rings			
Tree nº	Disc nº	Tree height (m)	Age (nº of rings)		Heartwood	Sapwood	Heartwood	Sapwood
1	1	0.1	49	49	29	20	28	21
	3	5.3	38	38	21	17	21	17
	5	10.5	35	35	18	17	18	17
	7	15.7	29	29	14	15	14	15
	9	20.8	23	23	11	12	11	12
	10	23.4	19	19	8	11	8	11
2	1	0.1	48	48	30	18	22	26
	3	5.4	42	42	25	17	28	14
	5	10.6	39	39	24	15	25	14
	7	15.7	29	29	17	12	17	12
	9	20.9	22	22	12	10	12	10
	11	26.0	15	15	7	8	7	8
3	1	0.1	48	48	30	18	29	19
	3	5.4	41	41	18	23	20	21
	5	10.6	36	36	16	20	17	19
	7	15.8	32	32	14	18	14	18
	9	20.9	26	26	12	14	12	14
	10	23.5	23	23	10	13	10	13
4	1	0.2	39	39	27	12	29	10
	3	2.8	35	35	22	13	23	12
	5	5.5	30	30	18	12	18	12
	7	8.1	25	25	15	10	14	11
	9	10.7	19	19	10	9	10	9
	10	13.3	15	15	7	8	7	8
5	1	0.2	49	49	28	21	34	15
	3	5.4	45	45	22	23	20	25
	5	10.6	37	37	19	18	17	20
	7	15.7	34	34	17	17	17	17
	8	18.3	34	34	20	14	20	14
	9	20.9	31	31	16	15	16	15
6	1	0.2	46	46	23	23	23	23
	3	5.4	44	44	20	25	20	25
	5	10.6	43	43	20	23	20	23
	7	15.8	40	40	17	23	17	23
	9	20.9	37	37	17	20	17	20
	11	26.2	34	34	16	18	16	18
7	1	0.1	46	46	31	15	28	18
	3	5.2	41	41	22	19	21	20
	5	10.4	36	36	17	19	17	19
	7	15.6	28	28	13	15	12	16
	9	20.7	23	23	10	13	10	13
	11	25.9	16	16	5	11	5	11

Site: Estrela

					Number of annual rings			
Tree nº	Disc nº	Tree height (m)	Age (nº of rings)		Heartwood	Sapwood	Heartwood	Sapwood
8	1	0.1	64	64	47	17	40	24
	3	5.3	52	52	32	20	33	19
	5	10.5	50	50	29	21	30	20
	7	15.7	45	45	27	18	26	19
	9	20.8	38	38	21	17	21	17
	11	26.0	23	23	11	12	11	12
9	1	0.1	53	53	36	17	38	15
	3	5.3	47	47	28	19	31	16
	5	10.5	44	44	29	15	29	15
	7	15.7	33	33	19	14	20	13
	9	20.9	26	26	15	11	15	11
	11	26.1	14	14	6	8	6	8
10	1	0.2	59	59	41	18	32	27
	3	5.3	49	49	17	32	21	28
	5	10.5	31	31	8	23	7	24
	7	15.7	29	29	11	18	11	18
	8	18.2	23	23	8	15	8	15
	9	20.8	18	18	6	12	6	12

Appendix II – Measurements of the radial width of heartwood, sapwood and bark of eight radii of the disc samples (as numbered in Appendix I) for each tree in the sites of Cabreira and Estrela

Site: Cabreira

		Disc nº					
		1			2		
Tree	Radii	Heartwood (cm)	Sapwood (cm)	Bark (cm)	Heartwood (cm)	Sapwood (cm)	Bark (cm)
1	R1	25.5	34.3	36.5	18.3	24.3	25.2
	R2	26.6	37.3	0.0	17.8	23.9	24.6
	R3	26.8	35.3	36.7	16.3	21.9	22.6
	R4	24.0	34.0	35.8	18.0	25.3	26.5
	R5	26.6	34.5	36.2	20.1	26.6	28.0
	R6	25.8	33.3	35.2	21.0	27.1	28.0
	R7	21.8	27.8	30.0	18.5	24.7	26.0
	R8	18.0	25.3	27.2	19.0	25.1	26.4
2	R1	33.0	41.0	45.0	19.3	25.7	27.0
	R2	31.0	41.3	0.0	20.4	26.6	27.8
	R3	27.0	41.3	0.0	20.0	27.1	28.2
	R4	26.6	35.9	39.6	23.5	29.3	30.5
	R5	29.8	41.8	46.2	22.7	29.7	31.0
	R6	35.0	49.6	53.2	24.6	31.5	33.0
	R7	36.3	50.2	0.0	23.5	30.0	31.0
	R8	38.0	48.3	50.8	19.0	24.9	27.4
3	R1	19.0	27.5	30.5	15.0	24.1	25.3
	R2	18.0	28.8	31.0	18.2	27.8	28.8
	R3	22.5	32.0	34.5	14.2	22.9	24.3
	R4	25.0	36.0	39.8	13.5	22.1	0.0
	R5	23.5	31.2	35.0	14.2	21.9	22.4
	R6	25.0	27.3	30.0	14.3	23.1	24.3
	R7	19.5	28.2	0.0	13.5	21.4	22.5
	R8	19.3	28.2	0.0	13.7	20.8	22.5
4	R1	22.8	30.0	33.0	15.9	22.2	23.3
	R2	21.4	28.0	31.5	17.3	24.6	25.9
	R3	22.3	30.2	34.5	22.0	28.4	29.3
	R4	26.3	30.3	36.0	18.9	24.9	26.0
	R5	26.0	30.2	34.0	17.3	23.5	24.5
	R6	24.2	30.5	32.3	17.1	23.0	24.1
	R7	21.6	32.0	35.7	15.1	20.7	21.7
	R8	18.8	26.5	30.0	14.7	20.1	21.2
5	R1	30.0	31.8	34.7	18.9	23.4	24.8
	R2	27.2	28.5	31.0	19.0	22.8	24.0
	R3	21.5	27.0	29.5	19.0	23.5	24.5
	R4	23.5	28.0	30.3	17.7	22.1	23.3
	R5	25.5	28.5	30.5	17.3	21.2	22.5
	R6	26.0	27.5	30.0	19.3	23.6	24.1
	R7	21.0	27.0	29.5	20.5	0.0	0.0
	R8	0.0	0.0	0.0	20.7	25.4	26.7

Site: Cabreira

		Disc nº					
		3			4		
Tree	Radii	Heartwood (cm)	Sapwood (cm)	Bark (cm)	Heartwood (cm)	Sapwood (cm)	Bark (cm)
1	R1	16.5	23.3	24.3	14.6	20.8	21.8
	R2	20.1	26.3	27.2	13.5	20.6	21.5
	R3	19.5	25.3	26.6	13.6	20.3	21.2
	R4	15.8	22.1	23.3	15.2	21.9	22.6
	R5	13.1	19.1	20.1	15.5	22.1	22.9
	R6	14.0	19.9	20.7	13.5	19.6	20.5
	R7	14.5	21.0	21.9	12.6	19.3	20.2
	R8	16.5	23.5	24.4	14.5	20.9	21.7
2	R1	17.1	24.3	25.6	19.0	27.2	28.0
	R2	21.5	28.6	29.9	18.5	26.6	28.0
	R3	22.6	30.9	32.0	18.4	26.0	27.0
	R4	21.9	28.3	29.3	16.0	22.2	23.8
	R5	20.4	27.8	29.0	14.7	24.5	26.0
	R6	18.0	25.6	27.0	15.8	25.6	26.7
	R7	16.2	23.5	24.6	18.0	27.6	28.5
	R8	16.0	24.0	25.0	16.4	25.5	27.0
3	R1	14.7	21.8	23.1	10.4	18.4	0.0
	R2	13.8	22.0	23.0	11.2	20.0	21.2
	R3	13.9	22.8	24.0	13.0	18.7	20.3
	R4	12.0	20.9	22.5	13.5	19.6	20.2
	R5	12.0	19.1	20.5	15.1	21.7	22.8
	R6	12.2	19.4	20.6	11.3	19.8	20.7
	R7	14.0	22.5	23.5	9.3	16.5	17.5
	R8	15.4	24.3	25.4	9.5	17.5	18.5
4	R1	17.0	23.4	24.6	12.5	17.5	18.5
	R2	16.8	23.8	24.3	11.8	16.7	17.5
	R3	20.1	24.8	26.0	14.0	18.9	19.8
	R4	16.7	21.5	22.6	14.6	19.4	0.0
	R5	14.5	20.1	21.3	13.2	18.8	19.7
	R6	14.0	19.6	20.9	13.4	19.2	20.0
	R7	15.5	22.2	23.2	14.2	20.7	21.6
	R8	16.0	22.4	23.4	13.5	19.1	20.5
5	R1	17.2	21.8	23.1	15.7	20.5	21.4
	R2	16.2	20.9	22.0	15.5	20.7	21.9
	R3	17.5	22.9	24.0	19.0	24.5	25.4
	R4	18.2	24.4	25.5	16.5	21.8	0.0
	R5	16.7	21.2	22.4	14.8	19.9	20.9
	R6	16.3	21.7	22.6	13.4	19.1	19.9
	R7	16.3	21.9	23.0	13.4	19.4	20.1
	R8	17.2	22.7	23.8	15.6	20.8	21.7

Site: Cabreira

		Disc nº					
		3			4		
Tree	Radii	Heartwood (cm)	Sapwood (cm)	Bark (cm)	Heartwood (cm)	Sapwood (cm)	Bark (cm)
6	R1	16.9	24.3	25.1	12.5	17.8	18.6
	R2	16.7	23.3	24.5	12.2	18.9	19.8
	R3	15.7	22.0	23.0	14.5	20.4	21.4
	R4	15.0	21.2	22.1	16.0	21.1	21.8
	R5	16.0	22.4	23.4	15.2	19.4	20.9
	R6	19.5	23.0	23.8	12.5	18.2	19.0
	R7	14.7	21.0	22.2	12.8	18.7	19.6
	R8	15.1	21.9	22.9	13.2	19.5	20.6
7	R1	19.0	26.2	27.3	15.5	20.4	21.6
	R2	18.6	25.5	26.7	15.5	20.9	22.1
	R3	18.5	24.5	25.5	16.5	22.4	23.4
	R4	18.9	25.0	26.3	20.2	25.3	26.6
	R5	18.0	23.6	24.3	21.5	29.5	30.8
	R6	17.7	23.5	24.2	22.0	28.9	30.0
	R7	18.8	26.4	27.5	17.5	23.9	24.6
	R8	20.2	27.5	28.8	15.5	20.9	22.0
8	R1	15.8	21.1	22.0	15.2	22.1	22.7
	R2	15.5	21.2	22.2	14.0	20.8	21.8
	R3	16.0	21.6	22.6	13.5	19.3	0.0
	R4	17.0	22.7	23.4	13.0	19.0	19.9
	R5	17.4	24.0	24.8	14.0	19.9	20.4
	R6	17.0	23.6	24.5	14.5	20.7	21.5
	R7	17.5	23.1	24.0	15.5	21.4	22.2
	R8	16.5	22.0	22.7	16.3	22.7	23.4
9	R1	16.3	21.0	22.2	16.3	21.8	22.7
	R2	17.0	22.1	23.2	15.1	19.5	20.4
	R3	18.9	25.5	26.4	14.2	18.4	19.2
	R4	17.5	22.6	23.7	14.0	17.9	18.9
	R5	18.1	23.4	24.4	15.9	20.0	21.0
	R6	18.2	23.9	25.2	16.8	21.8	22.5
	R7	18.1	22.2	23.3	15.9	20.5	21.4
	R8	16.6	21.1	22.2	16.4	20.8	21.6
10	R1	21.2	28.2	29.2	16.0	25.0	25.8
	R2	20.7	29.0	29.8	18.0	25.0	25.7
	R3	19.0	25.6	26.5	16.5	23.4	24.2
	R4	17.2	23.7	24.6	17.0	21.2	22.1
	R5	16.1	23.2	24.2	15.5	22.0	22.9
	R6	16.2	23.9	24.9	15.3	21.1	0.0
	R7	16.6	25.7	26.8	14.3	20.0	20.8
	R8	18.5	26.6	27.7	15.3	20.9	21.8

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		Disc nº					
		5			6		
Tree	Radii	Heartwood (cm)	Sapwood (cm)	Bark (cm)	Heartwood (cm)	Sapwood (cm)	Bark (cm)
1	R1	13.2	20.7	21.4	7.5	12.0	12.4
	R2	15.0	22.0	22.8	7.5	13.0	13.5
	R3	16.0	25.2	26.1	8.8	14.3	15.0
	R4	15.5	23.8	24.9	9.4	15.8	16.3
	R5	14.2	23.0	23.8	9.0	15.0	15.6
	R6	13.4	20.0	21.0	10.4	16.5	17.1
	R7	18.0	22.0	22.8	9.8	15.3	15.8
	R8	13.3	18.9	19.9	8.3	13.6	14.0
2	R1	14.6	23.3	24.6	14.7	20.5	21.5
	R2	14.5	21.6	22.8	13.0	20.8	21.6
	R3	15.1	0.0	0.0	13.5	20.8	21.6
	R4	16.0	22.2	23.4	14.5	20.2	21.6
	R5	16.6	24.2	25.3	15.0	21.1	22.0
	R6	17.0	24.5	25.5	15.5	21.9	22.7
	R7	16.0	23.4	24.4	14.6	20.6	21.6
	R8	14.5	23.4	24.4	14.5	20.9	21.8
3	R1	8.7	15.5	16.5	8.4	16.3	17.2
	R2	9.3	16.5	17.1	7.3	15.2	16.0
	R3	9.0	15.5	16.6	6.6	12.8	13.8
	R4	8.2	15.1	16.1	6.8	13.0	13.8
	R5	9.2	17.9	19.0	7.0	13.5	14.5
	R6	10.0	18.5	19.6	6.7	12.5	13.4
	R7	9.5	17.3	18.5	8.0	14.4	15.3
	R8	8.6	16.5	17.6	7.8	15.8	16.7
4	R1	10.2	16.5	17.4	10.7	17.0	17.6
	R2	10.0	15.4	16.2	10.5	17.1	17.7
	R3	9.5	14.2	15.0	8.4	14.8	15.9
	R4	10.3	15.2	16.1	7.9	13.5	14.2
	R5	12.0	17.0	17.9	8.6	14.5	15.0
	R6	15.0	20.4	21.2	8.8	14.1	14.9
	R7	12.7	19.2	20.0	9.8	14.8	15.5
	R8	11.8	17.4	18.2	9.4	14.7	15.4
5	R1	12.5	18.6	19.5	10.6	16.0	16.6
	R2	14.0	18.9	19.6	10.5	16.1	16.8
	R3	15.7	17.2	18.5	9.9	14.5	15.2
	R4	10.7	15.1	16.1	10.0	14.6	0.0
	R5	10.7	15.7	16.5	10.5	15.9	16.4
	R6	11.0	16.5	17.4	11.5	16.7	17.3
	R7	12.8	17.4	18.2	11.0	16.2	16.9
	R8	15.0	0.0	0.0	11.0	16.0	16.6

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		Disc nº					
		5			6		
Tree	Radii	Heartwood (cm)	Sapwood (cm)	Bark (cm)	Heartwood (cm)	Sapwood (cm)	Bark (cm)
6	R1	11.5	16.1	17.5	7.5	13.4	14.0
	R2	11.5	16.8	17.6	7.6	14.3	14.9
	R3	13.0	17.5	18.3	8.6	14.4	15.0
	R4	10.5	16.2	16.9	7.5	13.3	14.0
	R5	11.0	16.9	17.3	8.4	13.5	14.1
	R6	12.6	18.0	18.8	8.1	13.7	14.0
	R7	14.0	19.5	20.5	8.8	14.1	14.7
	R8	12.5	17.5	18.6	8.5	13.5	14.2
7	R1	13.2	18.2	19.2	13.6	20.6	21.4
	R2	16.0	22.4	23.5	14.5	21.8	22.7
	R3	18.5	25.6	26.5	14.5	20.5	21.4
	R4	18.0	25.0	26.0	13.0	18.7	19.4
	R5	15.5	21.8	22.9	12.2	17.7	18.8
	R6	15.0	20.4	21.5	12.4	17.4	18.4
	R7	14.5	19.4	20.1	11.6	17.6	18.5
	R8	13.6	19.0	19.8	12.5	18.9	19.8
8	R1	12.5	19.0	0.0	10.0	15.5	16.0
	R2	15.0	22.0	23.2	10.0	15.8	16.4
	R3	16.0	22.3	23.1	10.7	16.3	16.8
	R4	13.5	20.2	20.7	10.4	16.5	0.0
	R5	11.8	17.5	18.1	10.3	16.8	17.4
	R6	11.5	16.9	0.0	10.5	17.3	17.9
	R7	11.0	17.4	18.1	11.0	16.6	17.2
	R8	12.5	18.5	19.3	11.0	15.5	16.0
9	R1	12.7	17.1	17.8	11.4	16.0	16.8
	R2	12.4	17.1	17.8	11.3	16.1	16.9
	R3	12.9	17.3	18.0	10.2	14.7	15.2
	R4	13.0	17.8	18.5	9.9	14.1	14.8
	R5	12.8	17.3	18.2	10.5	15.0	15.6
	R6	12.8	17.3	18.1	10.3	15.0	15.6
	R7	13.3	17.5	18.2	10.6	14.8	15.5
	R8	13.5	18.4	19.0	11.1	15.4	16.0
10	R1	15.0	22.0	22.9	12.0	17.2	17.9
	R2	16.4	22.5	23.5	10.7	17.2	17.7
	R3	15.5	22.0	22.6	10.7	17.5	17.8
	R4	18.0	25.3	25.9	11.0	17.5	18.1
	R5	16.1	24.2	25.0	10.5	18.7	19.5
	R6	14.0	20.8	21.5	12.0	20.4	20.9
	R7	12.0	19.0	20.0	13.0	21.0	21.6
	R8	13.5	20.4	21.0	12.2	19.0	19.5

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		Disc nº					
		7			8		
Tree	Radii	Heartwood (cm)	Sapwood (cm)	Bark (cm)	Heartwood (cm)	Sapwood (cm)	Bark (cm)
1	R1	7.6	12.7	13.2	7.7	13.4	13.8
	R2	7.9	12.7	13.2	6.5	12.0	12.5
	R3	7.0	11.9	12.4	6.0	12.1	12.9
	R4	7.0	11.9	12.4	4.5	9.1	9.7
	R5	7.6	12.7	13.2	4.0	8.1	0.0
	R6	8.0	13.1	13.6	4.4	8.4	8.8
	R7	8.0	13.0	0.0	5.5	10.2	10.7
	R8	7.5	12.5	13.0	6.8	12.4	13.0
2	R1	11.9	17.8	18.4	10.3	15.4	16.1
	R2	12.4	17.5	18.4	11.1	16.6	17.3
	R3	13.4	19.2	19.8	11.0	17.9	18.6
	R4	12.0	18.4	19.3	10.3	16.1	17.0
	R5	11.8	17.9	18.8	9.5	16.0	16.6
	R6	12.1	17.6	18.5	9.5	15.7	16.5
	R7	11.2	18.1	19.0	10.0	16.1	16.8
	R8	12.0	18.5	19.4	11.7	16.2	17.0
3	R1	7.2	13.2	14.1	3.8	11.0	11.6
	R2	6.6	14.4	15.6	4.1	11.5	12.3
	R3	5.7	13.7	14.9	4.1	11.8	12.5
	R4	5.8	13.5	14.3	3.5	10.8	11.3
	R5	6.2	14.2	14.9	3.5	9.4	9.9
	R6	5.9	12.4	13.1	3.1	8.8	9.3
	R7	9.6	12.5	13.2	3.4	8.5	9.2
	R8	7.0	13.2	14.3	3.5	9.0	9.6
4	R1	7.0	13.5	14.1	4.0	8.7	9.4
	R2	7.2	13.1	0.0	4.0	8.6	9.2
	R3	6.7	12.3	12.9	4.0	9.3	9.8
	R4	6.5	11.3	12.0	4.4	10.4	10.9
	R5	6.9	11.6	12.3	5.2	10.3	10.8
	R6	7.0	12.2	13.0	4.9	9.4	10.0
	R7	7.0	11.8	12.4	4.5	8.9	9.5
	R8	7.0	12.2	12.9	4.1	8.8	9.5
5	R1	7.6	12.2	12.7	7.4	12.8	13.3
	R2	7.7	12.8	13.4	7.0	12.2	12.6
	R3	8.1	14.2	14.8	6.6	10.5	11.0
	R4	8.4	14.1	14.7	5.7	10.8	11.4
	R5	8.1	13.7	14.4	6.9	11.5	12.1
	R6	7.8	13.0	13.6	7.0	11.7	12.2
	R7	8.2	13.9	14.5	8.5	12.9	13.5
	R8	8.2	13.7	14.2	6.5	11.9	12.2

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		Disc nº					
		7			8		
Tree	Radii	Heartwood (cm)	Sapwood (cm)	Bark (cm)	Heartwood (cm)	Sapwood (cm)	Bark (cm)
6	R1	6.0	10.6	11.1	2.4	7.1	
	R2	5.8	9.9	10.4	2.4	7.1	7.4
	R3	5.3	10.0	10.5	2.4	7.1	7.6
	R4	4.9	10.3	10.8	2.5	7.1	7.5
	R5	4.7	9.8	10.3	2.6	7.7	8.1
	R6	4.4	9.9	10.3	2.6	7.9	
	R7	4.8	9.9	10.4	2.5	7.8	8.2
	R8	6.0	10.7	11.3	2.6	7.4	7.8
7	R1	12.5	19.4	20.4	8.4	14.8	15.5
	R2	12.7	18.7	19.5	9.7	15.6	16.3
	R3	12.0	17.5	0.0	9.5	14.8	15.5
	R4	11.5	16.6	17.5	9.2	14.4	15.5
	R5	13.0	17.2	17.6	7.2	11.8	12.3
	R6	11.3	16.0	16.8	8.0	12.5	13.0
	R7	11.1	18.0	19.2	7.8	13.4	13.9
	R8	11.7	18.9	20.0	8.5	14.1	14.6
8	R1	8.5	14.4	14.7	7.5	13.3	13.8
	R2	8.2	13.8	14.2	7.6	13.4	14.0
	R3	8.0	13.1	13.6	7.7	13.6	14.2
	R4	7.7	13.9	14.1	8.1	14.1	14.5
	R5	8.2	14.0	14.5	7.5	11.6	12.1
	R6	8.3	13.7	14.3	8.0	11.6	12.7
	R7	8.1	13.2	13.8	8.0	13.0	14.3
	R8	8.5	13.5	13.9	7.5	13.3	13.8
9	R1	8.1	12.1	12.7	5.7	9.9	10.2
	R2	8.5	12.7	13.3	6.5	10.3	10.7
	R3	8.1	12.5	13.1	6.6	11.0	11.5
	R4	8.5	13.1	13.6	6.7	11.1	11.7
	R5	8.5	12.8	13.4	6.0	10.0	10.6
	R6	8.6	12.5	13.1	5.4	9.2	9.7
	R7	8.8	13.1	13.6	5.5	9.5	10.0
	R8	9.1	13.3	13.9	5.7	9.9	10.3
10	R1	9.1	17.4	18.1	6.5	12.2	12.8
	R2	9.5	17.2	18.0	6.5	12.2	12.7
	R3	9.5	16.7	17.4	6.2	12.7	13.2
	R4	8.5	16.0	16.7	6.0	13.1	13.6
	R5	9.0	15.4	15.9	6.5	13.1	13.7
	R6	8.0	15.1	15.5	7.9	14.3	14.9
	R7	9.0	17.1	17.7	7.5	15.1	15.8
	R8	9.5	18.0	0.0	7.0	13.8	14.5

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		Disc nº					
		9			10		
Tree	Radii	Heartwood (cm)	Sapwood (cm)	Bark (cm)	Heartwood (cm)	Sapwood (cm)	Bark (cm)
1	R1	3.3	7.8	8.2	1.3	5.5	5.8
	R2	3.4	7.9	8.2	1.3	5.5	5.9
	R3	3.5	8.7	9.2	1.5	5.8	6.2
	R4	4.0	9.3	9.7	1.7	6.4	6.8
	R5	4.0	8.7	9.1	1.8	6.6	7.1
	R6	3.8	8.2	0.0	1.8	6.3	6.7
	R7	3.0	7.9	8.3	1.8	6.5	6.9
	R8	3.2	8.0	8.3	1.6	6.0	6.3
2	R1	9.2	15.2	16.0	4.3	9.9	10.3
	R2	9.3	16.2	16.7	5.1	10.4	11.0
	R3	8.1	14.2	14.8	5.4	11.3	11.8
	R4	9.1	14.7	15.3	5.4	11.4	12.0
	R5	8.5	14.2	15.0	5.4	11.1	11.5
	R6	9.1	15.0	15.6	5.3	10.4	11.0
	R7	9.1	14.6	15.3	5.0	10.2	10.6
	R8	10.3	14.9	0.0	4.7	10.3	10.8
3	R1	1.1	6.4	0.0	0.0	6.4	6.7
	R2	1.1	6.2	6.5	0.0	5.4	5.7
	R3	1.2	7.2	7.7	0.0	6.2	6.5
	R4	1.2	8.7	9.1	0.0	5.0	5.5
	R5	1.2	7.9	8.5	0.0	4.6	5.0
	R6	1.0	7.0	7.5	0.0	4.3	4.6
	R7	1.1	6.6	7.0	0.0	4.4	4.7
	R8	1.1	6.5	6.9	0.0	4.7	5.3
4	R1	2.5	6.4	6.8			
	R2	2.5	6.2	6.8			
	R3	2.7	6.3	6.8			
	R4	2.7	6.6	7.2			
	R5	2.5	7.2	7.7			
	R6	2.6	7.8	8.4			
	R7	2.4	7.4	7.8			
	R8	2.5	6.7	7.2			
5	R1	3.8	8.0	8.3	1.5	5.9	6.0
	R2	3.5	7.9	8.3	1.5	6.0	6.3
	R3	3.5	8.7	9.1	1.9	6.2	6.7
	R4	3.5	8.3	8.6	2.0	6.0	6.2
	R5	3.3	7.6	8.0	1.6	6.1	6.4
	R6	3.3	7.7	8.1	1.7	5.9	6.2
	R7	3.4	8.0	8.4	1.5	5.7	6.0
	R8	3.9	8.3	8.7	1.4	5.4	5.7

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		Disc nº					
		9			10		
Tree	Radii	Heartwood (cm)	Sapwood (cm)	Bark (cm)	Heartwood (cm)	Sapwood (cm)	Bark (cm)
6	R1	0.6	4.3	4.7			
	R2	0.7	4.6	4.9			
	R3	0.7	4.9	5.2			
	R4	0.6	4.5	4.8			
	R5	0.5	4.1	4.4			
	R6	0.5	4.1	4.5			
	R7	0.5	4.1	4.4			
	R8	0.5	4.1	4.4			
7	R1	5.7	10.3	10.8	2.6	6.9	7.3
	R2	5.8	10.9	11.4	2.6	6.8	7.4
	R3	6.4	10.4	11.2	2.7	6.9	7.3
	R4	6.4	11.3	11.8	2.7	7.1	7.4
	R5	6.8	11.6	12.1	3.0	7.6	7.9
	R6	6.8	11.5	12.0	3.2	8.3	8.7
	R7	5.6	10.4	10.8	3.0	8.0	8.4
	R8	5.0	9.6	10.1	2.8	7.5	7.9
8	R1	4.5	8.5	8.9	1.7	5.2	5.6
	R2	4.3	7.5	7.8	1.3	4.9	5.2
	R3	4.0	7.4	8.0	1.3	4.9	5.2
	R4	3.5	7.6	8.2	1.8	5.1	5.4
	R5	3.3	8.3	8.8	2.4	5.7	6.0
	R6	3.5	8.4	8.8	2.5	5.9	6.2
	R7	3.6	8.4	8.8	2.4	5.6	6.0
	R8	4.0	8.8	9.2	2.2	5.5	5.8
9	R1	4.0	7.1	7.5	1.5	4.2	4.4
	R2	4.2	7.5	7.8	1.5	4.1	4.3
	R3	4.0	7.0	7.5	1.4	4.2	4.4
	R4	3.5	7.2	7.6	1.5	4.3	4.6
	R5	3.4	6.7	7.1	1.7	4.7	5.0
	R6	3.7	6.5	6.8	2.5	5.9	6.1
	R7	3.5	6.7	7.1	2.0	4.6	5.1
	R8	3.6	6.8	7.2	1.8	4.5	4.7
10	R1	4.7	10.9	11.4	3.0	8.8	9.2
	R2	5.8	10.6	11.1	4.9	9.1	9.7
	R3	5.4	10.5	0.0	3.7	8.6	9.0
	R4	4.5	13.4	14.4	3.0	8.2	8.6
	R5	4.8	11.7	12.2	2.0	7.4	7.8
	R6	4.0	11.3	0.0	2.3	7.7	8.1
	R7	5.1	12.2	12.7	2.0	7.8	8.2
	R8	4.4	11.6	12.2	2.0	8.0	8.4

Site: Cabreira

		Disc nº					
		11			12		
Tree	Radii	Heartwood (cm)	Sapwood (cm)	Bark (cm)	Heartwood (cm)	Sapwood (cm)	Bark (cm)
1	R1		3.7	4.0			
	R2		3.7	4.0			
	R3		3.9	4.1			
	R4		4.0	4.2			
	R5		4.2	4.5			
	R6		4.2	4.4			
	R7		4.2	4.4			
	R8		4.0	4.2			
2	R1	2.8	7.7	8.1		5.1	5.4
	R2	3.1	8.0	8.5		4.6	5.1
	R3	3.2	8.5	8.9		4.5	4.9
	R4	3.0	8.2	8.6		4.8	5.2
	R5	2.8	7.7	8.1		4.6	5.0
	R6	2.8	7.8	8.1		4.8	5.2
	R7	2.9	8.0	8.2		5.2	5.6
	R8	2.8	7.5	7.9		5.4	6.1
3	R1		2.4	3.2			
	R2		2.1	2.3			
	R3		2.2	2.5			
	R4		2.2	2.4			
	R5		2.2	2.5			
	R6		2.5	2.8			
	R7		2.5	2.7			
	R8		2.5	2.7			
4	R1						
	R2						
	R3						
	R4						
	R5						
	R6						
	R7						
	R8						
5	R1		2.9	3.1			
	R2		2.9	3.2			
	R3		3.2	3.5			
	R4		3.2	3.4			
	R5		3.0	3.2			
	R6		2.9	3.2			
	R7		3.0	3.3			
	R8		2.8	3.1			

Site: Cabreira

		Disc nº					
		11			12		
Tree	Radii	Heartwood (cm)	Sapwood (cm)	Bark (cm)	Heartwood (cm)	Sapwood (cm)	Bark (cm)
6	R1						
	R2						
	R3						
	R4						
	R5						
	R6						
	R7						
	R8						
7	R1						
	R2						
	R3						
	R4						
	R5						
	R6						
	R7						
	R8						
8	R1						
	R2						
	R3						
	R4						
	R5						
	R6						
	R7						
	R8						
9	R1						
	R2						
	R3						
	R4						
	R5						
	R6						
	R7						
	R8						
10	R1	0.4	5.8	6.0		3.4	3.7
	R2	0.4	6.1	6.4		3.3	3.6
	R3	0.4	6.3	6.8		3.0	3.2
	R4	0.4	5.9	6.2		3.0	3.2
	R5	0.4	6.0	0.0		3.0	3.2
	R6	0.4	5.5	5.9		3.1	3.3
	R7	0.4	5.4	5.7		3.5	3.7
	R8	0.4	5.4	5.7		3.1	3.3

Site: Estrela

		Disc nº					
		1			3		
Tree	Radii	Heartwood (cm)	Sapwood (cm)	Bark (cm)	Heartwood (cm)	Sapwood (cm)	Bark (cm)
1	R1	18.5	7.1	2.1	14.2	6.2	1.0
	R2	23.5	4.6	1.6	14.0	6.1	1.0
	R3	26.0	6.6	2.0	15.0	5.5	0.9
	R4	26.0	8.8	1.5	16.0	6.4	1.2
	R5	27.5	8.3	2.5	18.0	5.1	0.8
	R6	24.5	10.0	2.5	18.5	6.7	1.0
	R7	23.0	4.8		18.7	5.2	1.0
	R8	17.0	5.4	2.1	16.5	5.7	1.0
2	R1	24.5	4.3	3.6	20.4	4.2	1.9
	R2	25.0	2.4	3.3	21.6	5.0	0.9
	R3	24.6	4.0	2.7	21.1	4.5	2.0
	R4	25.0	5.4	2.7	20.0	4.2	1.5
	R5	26.5	5.5	3.0	19.5	3.6	1.0
	R6	24.0	7.0	3.4	19.0	3.6	2.0
	R7	27.5	5.0	3.5	19.2	4.4	2.1
	R8	25.4	5.2		20.0	3.5	1.3
3	R1	21.5	5.4	3.0	15.2	7.0	1.2
	R2	20.0	4.9	2.5	16.0	7.5	2.0
	R3	19.5	6.7	2.5	17.0	7.3	1.5
	R4	21.0	8.2	2.2	17.0	5.3	1.5
	R5	20.5	8.0	2.0	15.9	6.5	1.4
	R6	20.0	6.0	2.3	18.0	5.2	1.5
	R7	18.0	5.1	2.6	15.5	6.8	1.3
	R8	19.0	7.2	2.5	17.0	5.0	1.5
4	R1	28.5	8.8		19.0	7.0	1.4
	R2	33.0			19.5	6.5	1.2
	R3	33.0	10.4		18.7	6.9	1.3
	R4	32.4	10.8		21.5	6.5	
	R5	31.0	14.6		24.2	5.5	1.2
	R6	32.0	9.8		22.5	6.9	1.5
	R7	42.6	9.4	2.5	23.5	6.0	1.4
	R8	35.0	7.4	5.0	23.0	8.4	1.3
5	R1	20.7	11.6	2.0	17.5	10.0	1.1
	R2	20.5	10.1	2.6	17.0	10.0	1.3
	R3	22.0	8.1	2.5	17.0	9.3	1.5
	R4	22.0	11.5	3.3	15.8	10.0	1.5
	R5	22.3	7.0	2.5	16.5	8.4	1.8
	R6	22.5	7.9	2.1	17.5	7.7	1.7
	R7	21.5	11.6	1.2	17.5	9.8	1.7
	R8	22.4	6.0	2.5	17.5	10.3	1.4

Site: Estrela

		Disc nº					
		1			3		
Tree	Radii	Heartwood (cm)	Sapwood (cm)	Bark (cm)	Heartwood (cm)	Sapwood (cm)	Bark (cm)
6	R1	27.3	10.5	2.5	16.5	8.4	1.5
	R2	23.0	14.0		16.0	10.0	1.5
	R3	24.0			16.0	11.4	1.5
	R4	19.5	9.6	2.4	19.0	9.9	1.7
	R5	16.5	8.5	2.6	18.3	11.0	1.7
	R6	18.5	7.6	2.4	18.5	11.7	1.5
	R7	23.0	7.3	2.5	20.0	7.8	1.7
	R8	26.0	12.2	3.0	17.5	10.0	1.5
7	R1	25.3	9.0	2.5	20.0	7.3	1.5
	R2	23.4	6.2		17.0	6.9	1.5
	R3	21.0	6.0		16.0	4.9	1.1
	R4	22.5	7.2		16.8	6.0	1.0
	R5	25.5	9.0		18.2	7.4	1.0
	R6	26.5	5.4	4.1	18.5	6.7	1.0
	R7	22.5	4.0	3.0	18.5	5.9	1.5
	R8	22.0	7.5	3.3	19.0	7.8	1.5
8	R1	18.5	5.5	2.0	17.5	4.0	1.0
	R2	20.0	4.7	2.0	17.0	4.5	1.1
	R3	20.0	6.7	1.6	17.0	4.5	1.0
	R4	21.5	6.3	1.7	17.0	5.0	1.1
	R5	21.0	5.7	3.0	17.0	4.4	1.2
	R6	20.0	4.8	3.2	17.3	5.0	1.3
	R7	19.5	4.3	2.3	17.5	5.3	0.9
	R8	21.0	4.0	2.0	17.0	4.0	1.2
9	R1	26.0	5.9	2.3	20.5	6.8	1.2
	R2	28.5	7.6	2.5	20.5	5.2	1.5
	R3	31.0	6.6	2.0	20.5	5.7	1.5
	R4	31.5	7.5	2.6	21.2	6.1	1.5
	R5	27.0	10.0	2.8	22.0	6.2	1.5
	R6	26.5	8.3		22.5	6.2	1.5
	R7	23.7	6.5	2.6	22.9	6.6	1.5
	R8	22.5	7.2	2.5	23.2	6.3	1.2
10	R1	21.0			16.0	12.1	1.1
	R2	23.0	7.5		17.0	8.3	1.3
	R3	27.0	8.3		17.0	7.1	1.5
	R4	23.5	3.6	2.5	15.5	7.8	1.6
	R5	20.5	7.5	3.6	15.5	8.1	1.1
	R6	16.5	6.2	2.6	15.0	8.1	1.3
	R7	17.0	7.5	2.0	14.5	7.5	1.5
	R8	19.0	6.2	2.8	14.0	7.8	1.5

Site: Estrela

		Disc nº					
		5			7		
Tree	Radii	Heartwood (cm)	Sapwood (cm)	Bark (cm)	Heartwood (cm)	Sapwood (cm)	Bark (cm)
1	R1	16.3	6.0	0.6	11.5	6.5	0.6
	R2	16.0	6.5	0.8	11.5	6.7	0.8
	R3	14.5	4.8	1.0	11.0	5.8	0.7
	R4	15.0	4.8	0.5	10.0	3.8	0.7
	R5	14.5	5.0	0.8	9.5	6.5	0.7
	R6	12.5	4.8	0.7	9.8	6.0	0.7
	R7	12.6	5.8	0.9	10.5	5.9	0.8
	R8	14.0	6.2	0.9	11.0	6.5	0.7
2	R1	18.5	3.6	1.4	13.0	4.4	1.0
	R2	17.9	4.3	1.2	13.0	6.2	1.0
	R3	16.5	5.0	1.5	13.5	5.4	1.0
	R4	16.0	5.5	1.5	13.2	5.3	1.1
	R5	16.5	4.8	1.5	13.0	4.4	1.2
	R6	18.0	4.0	1.5	12.5	4.5	1.0
	R7	18.2	5.2	1.2	12.5	3.8	1.0
	R8	18.0	3.6	1.4	13.0	3.8	1.0
3	R1	13.0	6.4	1.0	10.7	6.5	0.5
	R2	12.0	6.2	1.0	10.5	6.9	0.6
	R3	12.5	7.2	1.1	10.5	6.7	0.7
	R4	12.0	6.0	1.1	10.5	7.0	0.7
	R5	12.0	7.0	1.0	11.0	7.2	0.7
	R6	13.0	7.5	1.0	10.6	9.0	0.7
	R7	13.0	8.0	1.0	11.2	7.0	0.9
	R8	13.0			10.7	6.4	0.7
4	R1	17.5	9.0	1.0	12.0	6.5	1.2
	R2	21.0	7.5	1.5	13.0	6.5	1.0
	R3	20.5	6.5	1.5	12.5	7.0	1.1
	R4	16.5	6.7	1.1	14.2	6.0	1.1
	R5	15.5	6.8	1.3	14.0	7.1	1.0
	R6	15.5	6.8	1.1	13.5	6.2	1.0
	R7	16.3	7.5	1.3	12.6	6.2	1.1
	R8	17.2	8.2	1.0	13.0	6.5	1.0
5	R1	14.0	7.6	1.3	15.7	6.3	
	R2	14.0	8.5	1.3	13.5	7.9	
	R3	13.5	9.5	1.0	12.5	7.3	
	R4	13.4	9.8	1.0	12.5	8.0	0.8
	R5	14.0	8.3	1.3	13.5	8.0	1.0
	R6	16.0	7.0	1.0	13.0	9.0	0.9
	R7	16.0	6.3	1.1	13.5	7.9	
	R8	13.5	8.5	1.0	13.5	7.9	

Site: Estrela

		Disc nº					
		5			7		
Tree	Radii	Heartwood (cm)	Sapwood (cm)	Bark (cm)	Heartwood (cm)	Sapwood (cm)	Bark (cm)
6	R1	14.6	11.0	1.2	17.0	9.0	1.5
	R2	16.4	9.5	1.2	14.7	11.0	1.5
	R3	16.5	9.5	1.1	14.8	10.8	1.0
	R4	16.5	8.4	1.1	15.0	9.0	1.3
	R5	15.7	8.6	1.1	15.0	9.4	0.8
	R6	15.0	8.5	1.2	15.0	10.4	1.3
	R7	14.5	9.2	1.1	15.0	9.0	1.3
	R8	15.0			15.0	11.0	1.3
7	R1	12.7	7.0	1.1	10.0	7.7	1.0
	R2	13.9	6.5	1.2	9.0	7.4	0.7
	R3	15.0	6.4	1.1	9.5	6.5	1.0
	R4	15.5	7.7	0.9	11.5	8.5	1.0
	R5	17.2	9.2	1.0	10.8	8.3	0.8
	R6	16.8	9.6	1.0	12.0	8.4	0.8
	R7	14.6	8.6	1.1	12.5	8.6	1.0
	R8	13.3	6.3	1.1	12.5	8.3	1.0
8	R1	15.0	5.0	0.7	12.0	4.7	0.7
	R2	14.5	5.1	0.6	11.5	5.0	0.7
	R3	13.3	5.5	1.0	12.0	4.0	0.8
	R4	13.5	5.3	0.8	12.5	4.2	0.8
	R5	14.5	5.4	0.7	12.0	4.5	0.7
	R6	14.2	5.4	1.0	11.5	5.0	0.7
	R7	14.2	5.3	0.7	11.0	5.5	0.7
	R8	14.0	4.5	0.9	11.0	5.5	0.9
9	R1	16.5	5.0	1.1	13.5	6.2	0.7
	R2	17.0	7.0	1.0	14.5	6.7	0.7
	R3	18.5	7.0	1.0	15.0	7.6	0.8
	R4	21.0	7.7	1.5	15.5	7.5	1.0
	R5	22.0	8.3	1.0	14.7	7.1	0.7
	R6	21.0	8.0	1.0	14.0	6.8	
	R7	17.5	6.3	1.0	13.5	6.0	1.0
	R8	15.0	6.0	1.0	13.0	5.5	0.7
10	R1	12.5	6.7		7.5	11.0	1.0
	R2	13.5	9.2	1.0	8.0	8.2	1.1
	R3	13.5	8.8	1.0	8.0	8.0	1.0
	R4	12.0	9.4	1.1	7.0	9.5	
	R5	11.5	10.0	0.9	7.0	8.8	
	R6	11.0	9.4	1.5	7.5	9.2	
	R7	10.5	8.8	1.3	7.0	10.2	1.1
	R8	11.2	7.5	1.5	8.0	10.2	1.0

Site: Estrela

		Disc nº					
		9			10		
Tree	Radii	Heartwood (cm)	Sapwood (cm)	Bark (cm)	Heartwood (cm)	Sapwood (cm)	Bark (cm)
1	R1	6.5	7.2	0.6	3.8	5.1	0.5
	R2				4.2	5.3	0.4
	R3	6.0	7.2		4.0	5.4	0.5
	R4	6.0	6.6		4.0	5.4	0.5
	R5	7.0	5.3		4.1	5.5	0.4
	R6	6.5	6.0	0.5	4.3	5.8	0.5
	R7	7.0	6.8		4.3	5.7	0.5
	R8	8.4	5.8		3.8	5.6	0.4
2	R1	8.0	6.2	0.6	4.5	5.2	0.4
	R2	8.5	7.1	0.6	5.0	4.7	0.4
	R3	8.5	5.5	0.6	4.8	4.8	0.4
	R4	8.8	5.7	0.6	5.0	5.3	0.4
	R5	9.0	5.3	0.6	5.0	5.1	0.4
	R6	8.9	5.2	0.6	4.3	4.5	0.4
	R7	8.3	4.3	0.7	4.2	4.5	0.4
	R8	8.0	5.1	0.6	4.2	4.6	0.4
3	R1	7.7	7.0	0.9	5.9	6.8	0.4
	R2	8.2	6.1	0.7	5.7	7.5	0.4
	R3	8.3	5.9	0.7	5.8	7.3	0.5
	R4	8.0	5.7	0.7	5.9	6.5	0.6
	R5	7.6	6.2	0.8	6.0	5.9	0.5
	R6	7.5	7.0	0.7	5.7	6.3	
	R7	7.4	7.8	0.6	6.0	5.8	0.4
	R8	7.5	7.5	0.8	5.8	6.4	0.5
4	R1	7.8	6.3	0.6	4.3	6.2	0.5
	R2	7.0	6.4	0.5	5.0	6.0	
	R3	8.0	4.8	0.5	5.0	5.8	
	R4	7.5	5.8	0.5	5.2	5.9	
	R5	7.5	5.7	0.5	5.5	6.0	
	R6	7.1	5.8	0.5	5.7	5.5	0.4
	R7	7.0	5.7	0.6	5.5	5.3	0.3
	R8	8.0	5.7	0.5	5.0	5.8	0.4
5	R1	10.8	3.8	0.7	10.2	8.6	0.9
	R2	11.5	4.1	0.9	10.3	10.2	0.9
	R3	15.3	3.3	0.7	11.3	8.6	0.9
	R4	13.1	5.6	1.0	11.3	7.9	0.8
	R5	12.9	4.4	0.9	10.2	7.5	0.8
	R6	13.5	5.1	1.0	10.2	7.5	0.8
	R7	12.2	5.1	0.7	10.1		
	R8	11.6	4.0	0.6	9.9	8.1	0.9

Site: Estrela

		Disc nº					
		9			10		
Tree	Radii	Heartwood (cm)	Sapwood (cm)	Bark (cm)	Heartwood (cm)	Sapwood (cm)	Bark (cm)
6	R1	12.6	9.9	1.1	10.6	9.8	1.1
	R2	12.2	11.1	0.8	10.6	10.6	1.0
	R3	12.0	11.0	0.8	11.4	9.7	1.0
	R4	12.1	10.8	0.9	11.0	9.8	1.0
	R5	12.0	9.9	1.0	10.1	10.4	1.0
	R6	12.1	10.1	1.0	10.5	9.6	
	R7	11.8	10.0	1.1	9.6	10.3	1.0
	R8	11.6	10.6	1.0	10.5	9.6	0.9
7	R1	7.4	9.0	0.6	2.9	7.2	0.4
	R2	7.3	6.3	0.4	3.0	6.7	0.4
	R3	7.1	9.1	0.5	3.0	7.5	0.4
	R4	7.2	8.6	0.6	2.9	7.5	0.4
	R5	6.8	7.6	0.5	3.0	7.8	0.4
	R6	7.0	6.7	0.6	2.9	7.4	0.4
	R7	7.0	7.4	0.5	3.0	6.6	0.4
	R8	7.0	7.7	0.6	3.0	6.5	0.4
8	R1	8.3	5.2	0.6	5.0	5.1	0.4
	R2	8.2	5.7	0.5	4.8	4.8	0.2
	R3	8.1	5.4	0.5	5.2	4.6	0.3
	R4	8.6	5.7	0.5	5.4	4.0	0.3
	R5	9.0	5.9	0.6	5.4	4.6	0.4
	R6	9.3	5.0	0.6	5.5	4.8	0.3
	R7	8.4	5.2	0.5	5.2	4.9	0.4
	R8	8.0	5.0	0.5	5.0	5.1	0.4
9	R1	10.2	6.1	0.7	3.5	5.8	0.4
	R2	11.3	5.7	0.5	3.3	5.5	0.3
	R3	10.5	9.2	0.8	3.6	5.2	0.4
	R4	11.8	7.3	0.8	3.2	4.8	0.4
	R5	11.3	6.1	0.9	3.7	4.9	0.2
	R6	11.9	5.2	0.7	3.7	4.9	0.5
	R7	9.5	5.3	0.8	5.0	5.6	0.3
	R8	9.5	5.3	0.7	3.7	5.8	0.4
10	R1	4.6	9.3	0.4	2.5	7.5	0.5
	R2	4.5	8.8	0.4	2.5	7.4	0.3
	R3	4.5	8.4	0.4	3.0	6.5	0.4
	R4	4.6	8.0	0.5	3.0	6.8	0.4
	R5	4.5	7.6	0.5	3.1	7.1	0.4
	R6	4.5	7.8	0.5	2.8	7.2	0.4
	R7	4.5	7.9	0.4	3.0	7.7	0.4
	R8	4.5	8.9	0.4	3.0	10.7	0.4